PARASITOLOGY

LONDON
CAMBRIDGE UNIVERSITY PRESS
FETTER LANE, E.C. 4

CHICAGO: THE UNIVERSITY OF CHICAGO PRESS
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PARASITOLOGY

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VOLUME XXVII 1935

CAMBRIDGE
AT THE UNIVERSITY PRESS
1935

PRINTED IN GREAT BRITAIN

Medical
Taxon

CONTENTS

No. 1 (February)

PAGE

Lawrence, R. F. The Prostigmatic Mites of South African Lizards. (With 22 Figures in the Text) 1

Duke, H. Lyndhurst. On *Trypanosoma brucei*, *T. rhodesiense* and *T. gambiense* and their Ability to Infect Man . . . 46

Duke, H. Lyndhurst. Further Studies of the Behaviour of *T. rhodesiense*, recently Isolated from Man, in Antelope and other African Game Animals 68

Krull, Wendell H. Studies on the Life History of *Panopistus pricei* Sinitsin, 1931 [Trematoda]. (With Plate I, containing Figs. 1–7) 93

Leigh-Sharpe, W. Harold. *Epibrachiella impudica* (Nordmann) (Copepoda). (With 8 Figures in the Text) 101

Leigh-Sharpe, W. Harold. Some New and Rare Lernaeidae (Copepoda) from Plymouth. (With 2 Figures in the Text) . 107

Mellanby, Kenneth. A Comparison of the Physiology of the Two Species of Bed-bug which Attack Man. (With 3 Figures in the Text) 111

MacLeod, John. *Ixodes ricinus* in Relation to its Physical Environment. II. The Factors Governing Survival and Activity. (With 8 Figures in the Text) 123

No. 2 (May)

Taylor, E. L. Do Nematodes assist Bacterial Invasion of the Host by wounding the Wall of the Intestinal Tract? . . . 145

Rothschild, Miriam. The Trematode Parasites of *Turritella communis* Lmk. from Plymouth and Naples. (With 22 Figures in the Text) 152

Rothschild, Miriam. Note on the Excretory System of *Cercaria ephemera* Lebour, 1907 (*nec* Nitzsch). (With 7 Figures in the Text) 171

Skinker, Mary Scott. A Redescription of *Taenia tenuicollis* Rudolphi, 1819, and its Larva, *Cysticercus talpae* Rudolphi, 1819. (With 2 Figures in the Text) 175

Lapage, Geoffrey. The Second Ecdysis of Infective Nematode Larvae. (With 6 Figures in the Text) 186

Woodland, W. N. F. Some more Remarkable Cestodes from Amazon Siluroid Fish. (With Plates II–IV, containing Figs. 1–34, and Fig. I in the Text) 207

PAGE

WERTHEIM, P. A New Ciliate, *Entodinium bovis* n.sp., from the Stomach of *Bos taurus* L., with the Revision of *Entodinium exiguum, E. nanellum, E. simplex, E. dubardi dubardi* and *E. parvum*. (With 6 Figures in the Text) 226

SMITH, KENNETH M. and BALD, J. G. A Description of a Necrotic Virus Disease affecting Tobacco and Other Plants. (With Plates V, VI and 2 Text-figures) 231

BISHOP, ANN. Observations upon a "*Trichomonas*" from Pond Water. (With Plate VII) 246

KEILIN, D., TATE, P. and VINCENT, M. The Perispiracular Glands of Mosquito Larvae. (With 2 Figures in the Text) . . 257

BUXTON, P. A. Changes in the Composition of Adult *Culex pipiens* during Hibernation. (With 1 Figure in the Text) . . . 263

LEIGH-SHARPE, W. HAROLD. *Anchistrotos laqueus* n.sp. a Parasitic Copepod of *Serranus cabrilla*. (With 3 Figures in the Text) . 266

LEIGH-SHARPE, W. HAROLD. Two Copepods (*Lernaeenicus*) Parasitic on *Clupea*. (With 8 Figures in the Text) . . . 270

COLE, H. A. On some Larval Trematode Parasites of the Mussel (*Mytilus edulis*) and the Cockle (*Cardium edule*). (With 5 Figures in the Text) 276

THOMPSON, GORDON B. New Genera of *Mallophaga*. I. (With 4 Figures in the Text) 281

MELLANBY, KENNETH. The Structure and Function of the Spiracles of the Tick, *Ornithodoros moubata* Murray. (With 2 Figures in the Text) 288

EVANS, A. C. Studies on the Influence of the Environment on the Sheep Blow-fly, *Lucilia sericata* Meig. II. The Influence of Humidity and Temperature on Prepupae and Pupae. (With 6 Figures in the Text) 291

EVANS, A. C. Studies on the Influence of the Environment on the Sheep Blow-fly, *Lucilia sericata* Meig. III. The Influence of Humidity and Temperature on the Adult. (With 4 Figures in the Text) 299

No. 3 (JULY)

WOOLCOCK, VIOLET. Digenetic Trematodes from some Australian Fishes. (With Plates VIII and IX and 6 Figures in the Text) 309

KIRTISINGHE, P. Parasitic Copepods of Fish from Ceylon. (With 46 Figures in the Text) 332

THEILER, HANS, AUGUSTINE, DONALD L. and SPINK, WESLEY W. On the Persistence of Eosinophilia, and on Immune Reactions in Human Trichinosis, Several Years after Recovery . . 345

PAGE

SEN, S. K. The Mechanism of Feeding in Ticks. (With 15 Figures in the Text) 355

RAY, HARENDRANATH. On a New Coccidian, *Eimeria laminata* n.sp., from the Intestine of an Indian Toad, *Bufo melanostictus* Schneid. (With Plate X, containing Figs. 1–11) 369

SRIVASTAVA, HAR DAYAL. New Parasites of the Genus *Orientophorus* n.gen. (Family Fellodistomidae) from an Indian Fresh-Water Fish, *Clupea ilisha*. (With 4 Figures in the Text) . 374

HAPPOLD, FRANK CHARLES and STEPHENSON, DORA. The Toxicity of Bactericidal Substances for Flagellate Parasites, with Special Reference to their Application in the Isolation of *Leptomonas* Forms 383

HOLMES, FRANCIS O. Method of Chain Formation in *Herpetomonas bancrofti*. (With 9 Figures in the Text) 394

PATTEN, RUTH. The Life History of *Merocystis kathae* in the Whelk, *Buccinum undatum*. (With Plates XI–XIV, containing Figs. 1–56, and Figs. I–X in the Text) 399

MANTER, H. W. The Structure and Taxonomic Position of *Megasolena estrix* Linton 1910 (Trematoda) with Notes on Related Trematodes. (With 7 Figures in the Text) 431

IYENGAR, M. O. T. Two New Fungi of the Genus *Coelomomyces* Parasitic in Larvae of *Anopheles*. (With 5 Figures in the Text) 440

SMITH, KENNETH, M. Two Strains of Streak: A Virus Affecting the Tomato Plant. (With Plates XV and XVI) 450

SHARIF, M. On the Presence of Wing Buds in the Pupa of Aphaniptera. (With 2 Figures in the Text) 461

No. 4 (OCTOBER)

CARR FRASER, W. A. The Comparison of the Efficiency of Anthelmintics 465

JOHRI, L. N. On Cestodes from Burma. (With 4 Figures in the Text) 476

HOPKINS, G. H. E. Some Observations on the Bionomics of Fleas in East Africa 480

MACLEOD, JOHN. *Ixodes ricinus* in Relation to its Physical Environment. III. Climate and Reproduction. (With 2 Figures in the Text) 489

MARSHALL, J. F. and STALEY, J. Some Adult and Larval Characteristics of a British "Autogenous" Strain of *Culex pipiens* L. 501

BISHOP, ANN. Observations upon *Chilomastix* from *Bufo vulgaris*, with notes on *Chilomastix aulastomi*. (With Plate XVII, containing Figs. 1–32) 507

No. 4 (October) *continued*

PAGE

McIntosh, Allen. Description of a tick, *Ornithodoros coprophilus* n.sp., from Bat Guano. (With Plate XVIII and 2 Text-figures) 519

Smith, Kenneth M. and Doncaster, J. P. The Preparation of Gradocol Membranes and their Application in the Study of Plant Viruses. (With 7 Figures in the Text) 523

Pentelow, F. T. K. Notes on the Distribution of the Larvae and Pupae of *Simulium* spp. in the River Tees and its Tributaries. (With 1 Figure in the Text) 543

Buxton, P. A. The Effect of *Proteosoma* upon the Survival of *Culex* 547

Gwynn, A. M. and Hamilton, A. G. Occurrence of a Larval Cestode in the Red Locust *Nomadacris septemfasciata* Serv. (With 4 Figures in the Text) 551

Tate, P. The Larva of *Phaonia mirabilis* Ringdahl, Predatory on Mosquito Larvae (Diptera, Anthomyidae). (With 8 Figures in the Text) 556

Shute, P. G. The Value of the External Spine of the Harpago in Identifying Varieties of *Anopheles maculipennis*. (With Plate XIX) 561

Dobell, Clifford. Researches on the Intestinal Protozoa of Monkeys and Man. VII. On the *Enteromonas* of Macaques and *Embadomonas intestinalis* 564

Wilson, Charles Branch. A Parasitic Copepod, *Pupulina flores*, Re-described, after Forty Years. (With 13 Figures in the Text) 593

Index of Authors 598

Index of Subjects 601

VOLUME XXVII FEBRUARY, 1935 No. 1

THE PROSTIGMATIC MITES OF SOUTH AFRICAN LIZARDS

BY R. F. LAWRENCE, PH.D.

Assistant in Charge of Arachnida, South African Museum, Cape Town

(With 22 Figures in the Text)

CONTENTS

PAGE

I. Introduction 1
II. Genera of prostigmatic mites from South Africa 3
III. Mites parasitic on Zonuridae 4
(1) *Zonurobia* n.gen. 4
Z. cordylensis n.sp. (p. 5); *Z. polyzonensis* n.sp. (p. 7); *Z. circularis* n.sp. (p. 8); five varieties of *Z. circularis*; *Z. semilunaris* n.sp. (p. 11); *Z. debilipes* n.sp. (p. 13); *Z. transvaalensis* (p. 15); *Z. sanguinea* n. sp. (p. 16); *Z. subquadrata* n.sp. (p. 18); *Z. montana* n.sp. (p. 19); key to the species of *Zonurobia* (p. 20)
(2) *Scaphothrix* n.gen. 20
S. convexa n.sp. (p. 21)
(3) *Ixodiderma* n.gen. 22
Remarks on the genus *Ixodiderma* 23
Ixodiderma inverta n.sp. (p. 24); *Ixodiderma lacertae* n.sp. (p. 29); *Ixodiderma pilosa* n.sp. (p. 30); key to the species of *Ixodiderma* (p. 32)
The mechanism of attachment in *Ixodiderma* 32
IV. Mites parasitic on Gerrhosauridae 33
(4) *Pterygosoma* Peters 33
Pterygosoma gerrhosauri n.sp. (p. 33); *P. bicolor* n.sp. (p. 34); *P. hystrix* n.sp. (p. 37); key to species of *Pterygosoma* occurring on Gerrhosauridae (p. 38)
V. General discussion 38
(*a*) Mite parasitism in relation to lepidosis 39
(*b*) The correlation of parasite and host in the family Zonuridae 41
(*c*) List of prostigmatic mites parasitic on South African Zonuridae and Gerrhosauridae 43
References and further papers bearing on the subject 45

I. INTRODUCTION

THE following paper as a preliminary study of the acarine parasites of South African lizards attempts to furnish as complete a list as possible of the prostigmatic mites of two families of lizards peculiar to South Africa, the Zonuridae and Gerrhosauridae.

The material has been derived from a large number of preserved specimens in the reptile collections of the South African Museum, the Albany Museum, Grahamstown, and the Transvaal Museum, from numerous living specimens

taken in the field and from captive lizards in the Cape Town Snake Park. The fact that many mites in a good state of preservation have been taken from reptiles which have lain for years in spirit, demonstrates the possibility of these parasites being adequately studied in centres far removed from the sources of the collection itself. The lacertilian collections in the museums of Europe and the United States should prove on investigation to be rich storehouses of acarine material. I wish here to express my warm thanks to the following interested helpers: Mr B. Peers, Curator of the Snake Park, for the careful examination of all lizards that passed through his hands and for his constant supplies of material; the Rev. K. Tasman for his collections from lizards in Rhodesia; the Directors of the various South African Museums; Prof. Alan Stephenson and Anne Stephenson for valuable assistance in the loan of a drawing apparatus; Mr J. Hewitt for suggestions and advice especially with regard to the systematics of South African lizards.

As the present paper is designed to give a presentation of the knowledge of lacertilian parasites both from the point of view of the systematics and distribution of the hosts themselves as well as of the parasites, it will be appropriate here to give a very general account of the South African lacertilian fauna with regard to its acarine parasites.

The lizard fauna of South Africa consists of nine families, the Geckonidae, Agamidae, Zonuridae, Scincidae, Lacertidae, Varanidae, Gerrhosauridae, Amphisbaenidae, and Chamaeleontidae, of which the Zonuridae and Gerrhosauridae[1] are endemic families while the remainder have a more or less wide distribution outside South Africa. Acarine parasites exclusive of adult and larval ticks have been found on six of these, none having been discovered on the Varanidae, Amphisbaenidae, or the chamaeleons; the two members of the family Varanidae, *Varanus niloticus* and *V. albigularis*, while not attacked by mite parasites are the specific hosts of the Ixodid, *Aponomma exornatum*. The acarine parasites of the remaining six families, exclusive of sporadic adult and larval Ixodidae, consist of two suborders which are phylogenetically and morphologically widely divergent, the Mesostigmata (Gamasoidea) and the Prostigmata; from an examination of numerous members of these six families it appears that Prostigmata occur only on certain families while Mesostigmatic mites are in general restricted to the remaining ones; thus the Prostigmata parasitise the four families Geckonidae, Agamidae, Zonuridae, and Gerrhosauridae, while Mesostigmata are found on the Lacertidae and Scincidae. Though, broadly speaking, this is true, certain exceptions should be noted; the Gerrhosauridae harbour parasites belonging to both the suborders and in this respect have an element in common with both groups; the three Lacertid genera *Tropidosaura*, *Eremias*, and *Scapteira*, are parasitised by a single species of Prostigmatic mite, *Ixodiderma lacertae*, the genus *Ixodiderma* being, however, far more common on Zonurid lizards. In spite of these exceptions I have found that throughout the large collections examined, containing all the known

[1] Two genera are found in Madagascar.

species of South African lizards and comprising more than 5000 individuals, the general correlation of host to parasite, as tabulated below, holds good.

AA.	Geckonidae Agamidae Zonuridae	Prostigmatic mites
AB.	Gerrhosauridae	Prostigmatic and Mesostigmatic mites
BB.	Lacertidae Scincidae	Mesostigmatic mites
CC.	Varanidae Amphisbaenidae Chamaeleontidae	Parasitic mites not present

I have not taken into account the temporary parasitism of the genus *Thrombicula*, the larval members of which attack both warm and cold-blooded vertebrates. M. André in 1929 described *T. agamae* from *Agama stellio* in Palestine, and I have no doubt that the South African Agamidae and probably many of the other families of lizards, are similarly infested.

II. GENERA OF PROSTIGMATIC MITES FROM SOUTH AFRICA

The prostigmatic mites of lizards were first studied by Peters in 1849 who erected the genus *Pterygosoma* to receive a parasite of *Agama mossambica*. Mégnin in 1878 created a second genus *Geckobia* for the reception of a mite from *Tarentola*, a Geckonid lizard, which Trägardh in 1905, together with a new form *Eupterygosoma*, retained in his paper with subgeneric rank, while contributing a third genus *Pimeliaphilus* from a Geckonid lizard *Platydactylus*; in the same year Banks contributed the genus *Geckobiella* from an Iguanid lizard for a form differing rather widely from the old world genera of prostigmatic mites; Hirst in 1926 included all these genera in his systematic paper on the group, where he rightly raises *Geckobia* to the status of a full genus, unites *Eupterygosoma* with *Pterygosoma* and includes Berlese's (1920) genus *Hirstiella* from a host unknown. Finally Vitzthum in 1931 incorporated these five genera in a separate family, the Pterygosomidae, and I have regarded them as members of this family in this paper.

The genera of Pterygosomidae which have been or are likely to be recorded from South African lizards are therefore the following: *Pterygosoma* from Agamidae and Gerrhosauridae, *Geckobia* and *Pimeliaphilus* from Geckonidae; to these I add three new genera described in the following paper found almost exclusively on Zonurid lizards: *Zonurobia*, *Scaphothrix*, and *Ixodiderma*. These genera can be distinguished by means of the following key.

Key to the South African genera of Pterygosomidae

1. A dorsal scute present **2**
   A dorsal scute not present **3**
2. Dorsum with few hairs, these very long ... ... **Pimeliaphilus**
   Dorsum with numerous hairs, these shorter ... ... **Geckobia**

3. Apex of hypostome much enlarged, dorsum with few hairs **4**
   Hypostome subparallel, dorsum with numerous hairs **5**
4. Body longer than wide, size large, skin leathery... ... ... **Ixodiderma**
   Body much wider than long, size small, skin not leathery ... ... **Scaphothrix**
5. Dorsum with a dense anterior patch of hairs on each side of the mouth-parts, eyes absent ... ...**Pterygosoma**
   Dorsum without a dense anterior patch of hairs on each side of the mouth-parts, eyes present ... ... **Zonurobia**

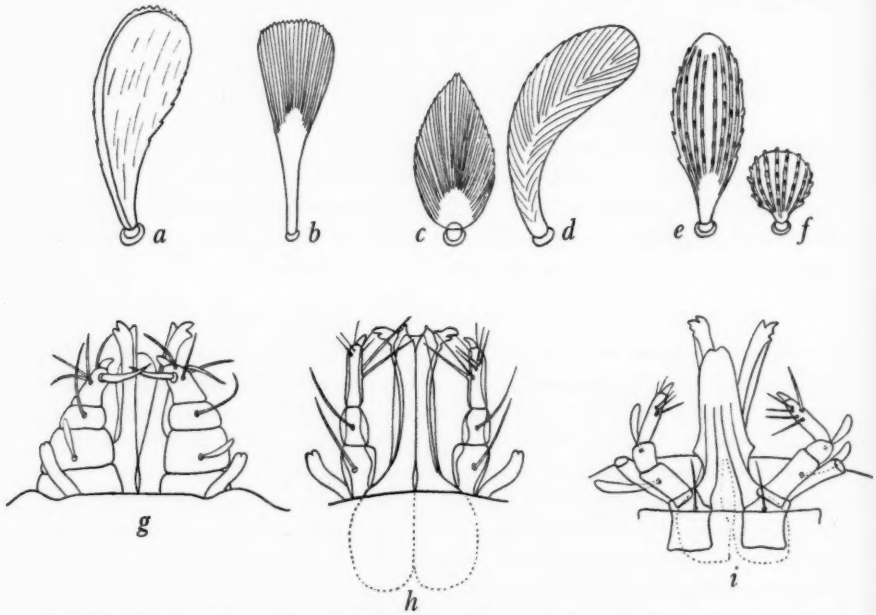


Fig. 1. Body hairs of *Zonurobia*: *a*, peripheral hair of *Z. cordylensis*; *b*, the same of *Z. polyzonensis*; *c* and *d*, dorsal and peripheral hairs of *Z. circularis*; *e* and *f*, posterior and anterior dorsal hairs of *Z. semilunaris*. Mouth-parts of *Zonurobia*: *g*, mouth-parts of *Z. polyzonensis*, dorsal view; *h*, the same of *Z. cordylensis*, dorsal view; *i*, the same of *Z. debilipes*, ventral view.

III. MITES PARASITIC ON ZONURIDAE

(1) *Zonurobia* n.gen.

(Fig. 1)

Body usually broader than long, covered dorsally with numerous more or less evenly distributed apically enlarged hairs, these hairs with various types of sculpture or with small sharp spicules, Fig. 1 *a–f*; margins of body with serially arranged paddle-shaped hairs usually larger than those of the dorsal surface; dorsal scute absent; eyes present, very small, situated near the anterior margin some distance laterally to the mouth parts; mouth-parts ventrally with a thin transparent plate covering their bases provided with a short spine on each side, palps normal, hypostome subparallel and flattened;

anal field* usually situated on ventral surface, the lateral folds with modified flattened hairs or spines or both; coxae of legs I and II completely fused forming a triangular common coxa with 2–3 spines, coxae of legs III and IV similarly with 1–2 spines, remaining segments of legs except the last usually with distally expanded hairs.

Nymph with fewer larger hairs on the dorsum; eyes larger than in adult situated on a smooth oval plaque in association with an apically enlarged hair; peritremes of stigmata well developed; mouth-parts, anal field, legs as in adult.

Larva with large oval hairs on dorsum; eyes as in nymph but larger; peritremes of stigmata and tracheal system developed; anal field without hairs; mouth-parts, legs as in nymph and adult.

Type and genotype, *Zonurobia cordylensis*.

Zonurobia cordylensis n.sp.

(Figs. 1 *a*, *h*, 2)

Colour in life light reddish brown to dark brown, spirit specimens darker.

Adult as in Fig. 2*a*, the body however often a little broader than in this figure; margins of the body with 12–15 paddle-shaped transparent hairs, these not including a group of 4–5 laterally to the anal field; dorsal surface with 40–45 hairs on each half, the areas near the sides less thickly covered and a median trapezoid area free of them, these hairs slightly smaller than the peripheral hairs; ventral surface with about seven hairs on each side of its posterior half, the four anterior ones considerably smaller than the remainder; anal field, Fig. 2*d*, flanked on each side by a longitudinal row of five blade-like hairs, the anterior ones sharp pointed and lanceolate; beneath these hairs three (sometimes two) small cylindrical spines on each side.

Mouth-parts, Fig. 1*h*, unusually long and slender, hypostome concavely notched at its apex, second and third joints of palp with a cylindrical spine-like hair in the middle above, these hairs not or only slightly serrated distally.

Legs. Anterior common coxa with two stout spines, one near its posterior apex and one distally near its articulation with the trochanter of leg I, posterior common coxa with only one spine at its posterior apex; trochanter I with a flattened hair on its anterior surface, trochanters II–IV similarly with a short spine, legs otherwise as in Fig. 2*a*, seen from above.

Nymph light in colour, almost transparent; dorsal surface as in Fig. 2*b*, ventral surface with 2–3 peripheral hairs laterally to the anal field, a row of five hairs on each side of the anal field, two flattened hairs and a few spines on each side of the middle line anterior to the anal field; legs with spines and hairs as in adult, mouth-parts as in adult.

Larva as in Fig. 2*c*, seen from above, no flattened hairs on ventral surface; anterior common coxa spined as in nymph and adult, coxa of leg III ill-defined and without a spine; mouth-parts as in nymph and adult.

* The term "anal field" is used in a topographical sense only; presumably the excretory pore and genital duct both open here.

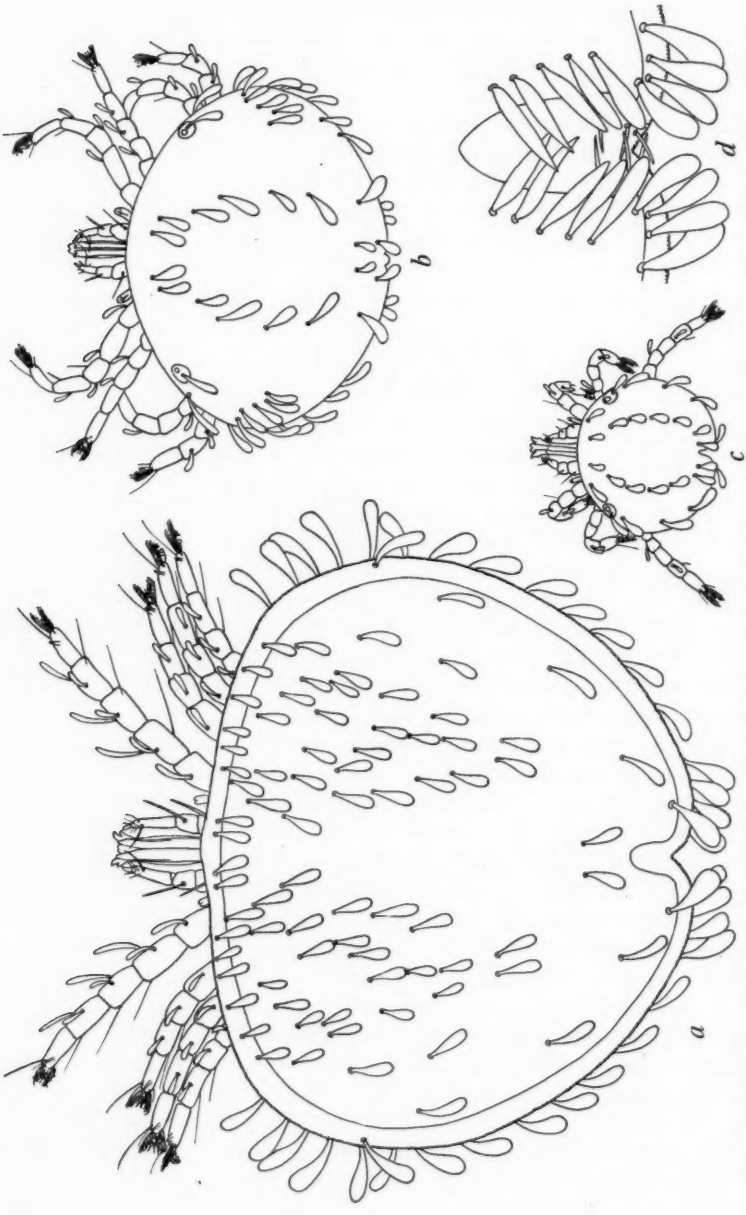


Fig. 2. *Zonurobia cordylensis*. *a*, dorsal view of adult; *b*, dorsal view of nymph; *c*, dorsal view of larva; *d*, anal field of adult, ventral view.

Dimensions. Adult, length 0·9 mm., breadth 1·1 mm.; nymph, length 0·5 mm., breadth 0·64 mm.; larva, length 0·26 mm., breadth 0·3 mm.

Types[1]. Seventy specimens from *Zonurus cordylus*, Table Mountain. This species occurs only on *Z. cordylus*; I have taken it from hosts recorded from East London, Stutterheim, George, Hogsback, in the Cape Province, and Smithfield in the Orange Free State. The specimens from the last-named locality perhaps constitute a variety; they are proportionately much broader than in the type with slight differences in the arrangement, size, and number of the hairs; the anal region and the mouth-parts on the other hand are essentially the same in the arrangement and structure of their spines and hairs.

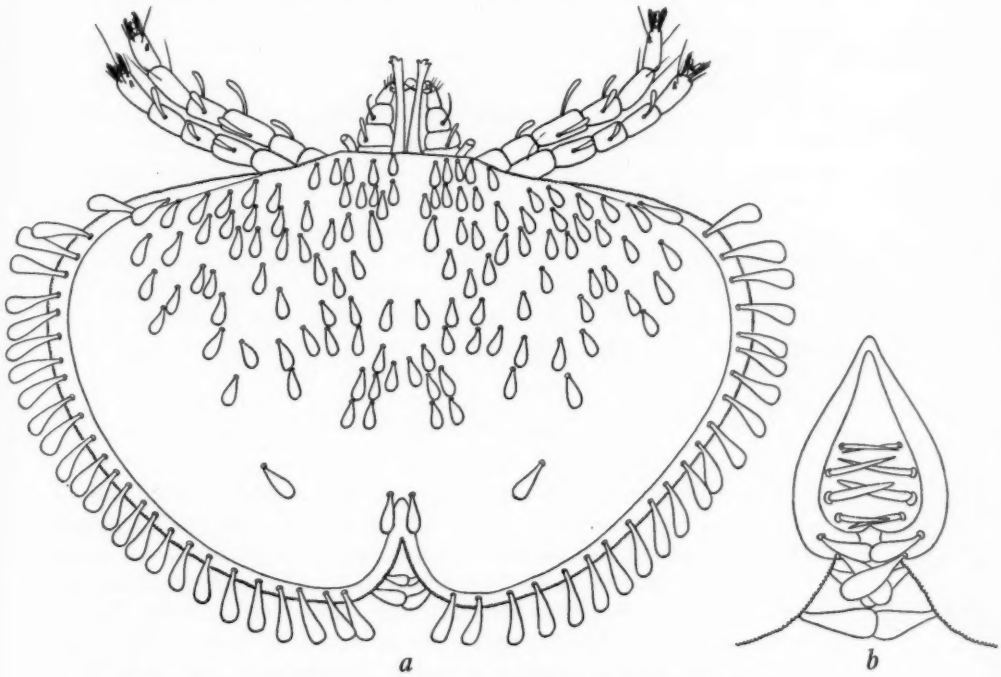


Fig. 3. *Zonurobia polyzonensis.* Adult. *a*, dorsal view; *b*, anal field, ventral view.

Zonurobia polyzonensis n.sp.

(Figs. 1*b*, *g*, 3)

Colour in life dull reddish brown, spirit specimens varying from light to dark brown.

Body as in Fig. 3*a*, considerably broader than long; periphery of body with 21–25 rather slender flattened paddle-like hairs (Fig. 1*b*), dorsal surface with about 60–70 hairs on each side, the areas at the sides and posteriorly more or

[1] Types except where otherwise mentioned are deposited in the South African Museum.

less free of hairs, these hairs somewhat smaller than the peripheral hairs; ventral surface with only 2–3 hairs on each side, anal field as in Fig. 3*b*, very large and horseshoe-shaped in living specimens.

Mouth-parts as in Fig. 1*g*, seen from above (the mandibles are intentionally drawn as if they were situated inferiorly to the palps in order to show the spination of the latter); segment II of palp dorsally with a short, slightly flattened and serrated hair, segment III with a long cylindrical spine, segment IV with a conspicuous spine below the claw on the mesial side, very stout basally but tapering sharply; hypostome rounded at its apex.

Legs. Anterior common coxa with three spines, one basal and one near each articulation with trochanters I and II, posterior common coxa with one basal spine only, legs otherwise armed as in Fig. 3*a*.

Dimensions. Length 0·66 mm., breadth 1 mm.

Types. Eighteen specimens from *Zonurus polyzonus*, the locality of the hosts unknown. I have taken numerous specimens from this host species recorded from Kimberley, Vondeling, Darling, Leliefontein (Namaqualand), Kuruman, Fourteen Streams, Van Rhynsdorp, and also four specimens from *Z. peersi* recorded from Kamiesberg (Namaqualand). The distribution of the latter host species is limited to certain parts of Namaqualand, where it is overlapped by that of *Z. polyzonus*; Mr B. Peers tells me that the two species live together in the same local areas though on different rock formations; it therefore seems probable that the four specimens taken from *Z. peersi* may be stragglers from *polyzonus*, and I prefer to regard the latter species as the true host of this scale mite.

Zonurobia circularis n.sp.

(Figs. 1*c*, *d*, 4)

Colour in life dark reddish brown, spirit specimens light to dark brown.

Body as in Fig. 4*a*, almost circular; margins of body with 18–22 regularly spaced medium-sized club-shaped hairs not including a group of 2–3 laterally to the anal field from which they are separated by a short space; almost the whole of the dorsal surface covered thickly with small scale-like hairs, Fig. 1*c*, differing from and smaller than the peripheral hairs, those in the middle of the anterior half of the body touching or almost touching each other; these hairs with bluntly pointed apices which are distinctly narrower than their bases. Ventral surface quite devoid of hairs except for one small slightly flattened hair at the upper level of the anal field between it and the side but a little nearer to the anal region, in addition a pair of spines nearer to the middle; anal field as in Fig. 4*b*.

Mouth-parts as in Fig. 4*a*, segment II of palp with a greatly expanded rounded hair, segment III with a much longer cylindrical hair slightly flattened apically, segment IV with a strong swollen spine on its medial side below the claw tapering suddenly in its apical half; hypostome notched apically.

Legs. Anterior common coxa with one basal spine and one spine each

below its articulation with trochanters I and II, posterior common coxa similar but lacking a spine below trochanter IV; segments of legs with expanded rounded hairs rather similar to that on segment II of the palp.

Dimensions. Length 0·74 mm., breadth 0·7 mm.

Types. Forty specimens from *Platysaurus guttatus*, Salisbury, Rhodesia. Other records: from *P. guttatus*, Plumtree, Chisawasha, Zimbabwe (Southern

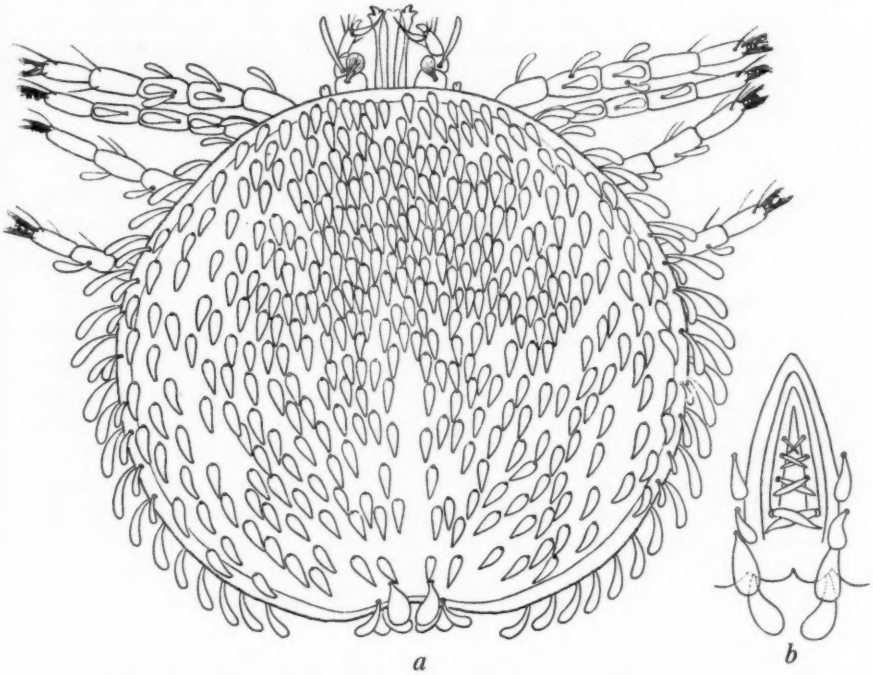


Fig. 4. *Zonurobia circularis*. Adult. *a*, dorsal view; *b*, anal field, ventral view.

Rhodesia) and Pietersburg, Mariepo, Shiliowane, Nylstroom (Eastern Transvaal). Four specimens from *Zonurus jonesi* probably constitute a variety, these specimens differing from the above description in having the peripheral hairs fewer in number and the dorsal hairs fewer and more scattered; in all other respects, however, they agree with the type, so that until more material can be examined I prefer to leave them under the above heading.

The following five varieties of *Zonurus circularis* can be distinguished:

Zonurobia circularis var. **latior** n.var.

The only constant difference between this form and the types of *Z. circularis* found on *Platysaurus guttatus* is in its greater width, the body being oval rather than round; this difference is at once apparent when a number of mites of both

forms are seen with the naked eye; the hairs of the dorsal surface appear to be fewer and more scattered than in the type; in the latter the hairs are usually in contact or overlap in the middle of the dorsal surface, while in the above variety they seldom do. With regard to other characters such as the spination of the mouth-parts, the anal field, and the number of peripheral hairs, the similarity in the details of the first two structures is so close that I feel it would be unwise to draw conclusions from minute differences which also occur throughout a large series of mites taken from the same host species; the number of the peripheral hairs in this as in other species can only be taken as approximate, and small differences in this number cannot be relied on as a character of systematic value.

Dimensions. Length 0·66 mm., breadth 0·83 mm.

Types. Twenty-five specimens from *Zonurus vittifer*, Belfast, Transvaal. Other specimens from the same host from Potgietersrust, Haenertsburg, Middelburg, Transvaal, and Ubombo, Zululand; from *Z. breyeri*, Waterberg Mountains, Transvaal.

Zonurobia circularis var. **spiniventer** n.var. (Fig. 5).

This variety differs more markedly from the type in the structure of the anal field than any of the other varieties; the most anterior of the three flattened hairs at the sides of the anal opening is here replaced by a spine, while another pair of spines is situated near the apex of the anal field; the flattened hair between the apex of the anal field and the lateral margin is here absent; more toward the middle of the ventral surface there is a pair of widely separated spines and just anterior to these a pair of narrowly separated spines. The number of the peripheral hairs and the spination of the mouth-parts is similar to that of the type, but the peripheral and dorsal flattened hairs of the body are much more weakly sculptured, the ribbing of these hairs being practically absent.

Dimensions. Length 0·56 mm., breadth 0·6 mm.

Types. Seventy specimens from *Platysaurus guttatus minor*, Nylstroom, Transvaal; other specimens from the same host, Waterberg District, Transvaal.

Zonurobia circularis var. **capensis** n.var.

This variety differs from the type only in the hair between the apex of anal field and lateral margin of the body being replaced by a spine; the hairs of the dorsum are a little more strongly sculptured.

Dimensions. Length 0·6 mm., breadth 0·6 mm.

Types. Thirteen specimens from *Platysaurus capensis*, Kamieskroon, Namaqualand.

Zonurobia circularis var. **longipilis** n.var.

Differing from the type in the anterior peripheral hairs just posterior to leg IV being almost twice as long as the most posterior ones; peripheral hairs diminishing regularly in length posteriorly; the hairs of the dorsum more

strongly sculptured than in the type, the ribs of these hairs much more clearly defined and fewer in number.

Dimensions. Length 0·48 mm., breadth 0·56 mm.

Types. Seventeen specimens from *Platysaurus guttatus*, Blyde River, Eastern Transvaal.

Zonurobia circularis var. **transvaalensis** n.var.

Differs from the type in having fewer and longer peripheral hairs which are about twelve in number; the hair between the apex of anal field and the lateral margin of the body considerably nearer to the former than in the type; hairs of dorsal surface more strongly sculptured and with fewer ribs than in the type.

Dimensions. Length 0·56 mm., breadth 0·56 mm.

Types. Eight specimens from *Platysaurus wilhelmi*, Olifants River, Eastern Transvaal.

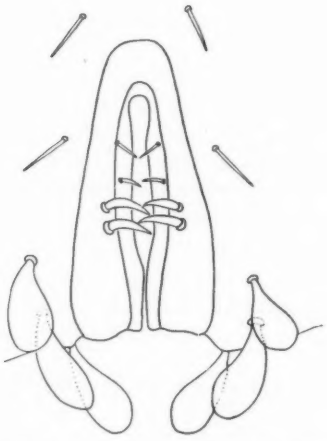


Fig. 5. *Zonurobia circularis spiniventer.* Anal field, ventral view.

Zonurobia semilunaris n.sp.

(Figs. 1*e,f*, 6)

Colour of spirit specimens light brown.

Body seen from above as in Fig. 6*a*, about twice as broad as long, the posterior margin always more convex than the anterior; dorsal surface covered with flattened hairs increasing progressively in size posteriorly, those nearest the peripheral hairs almost if not equal to them in size; peripheral hairs 14–16 in number inserted close to the posterior margin of the ventral surface; ventral surface as in Fig. 6*b*, characterised by two long almost cylindrical hairs on each side of the anal field and laterally to these one or two oval hairs; anterior to the anal field about 24–30 hairs arranged in three very irregular transverse rows, these distinctly smaller than the remaining hairs of the ventral surface; anal field as in Fig. 6*b*, in its posterior half three pairs of spines directed inwardly,

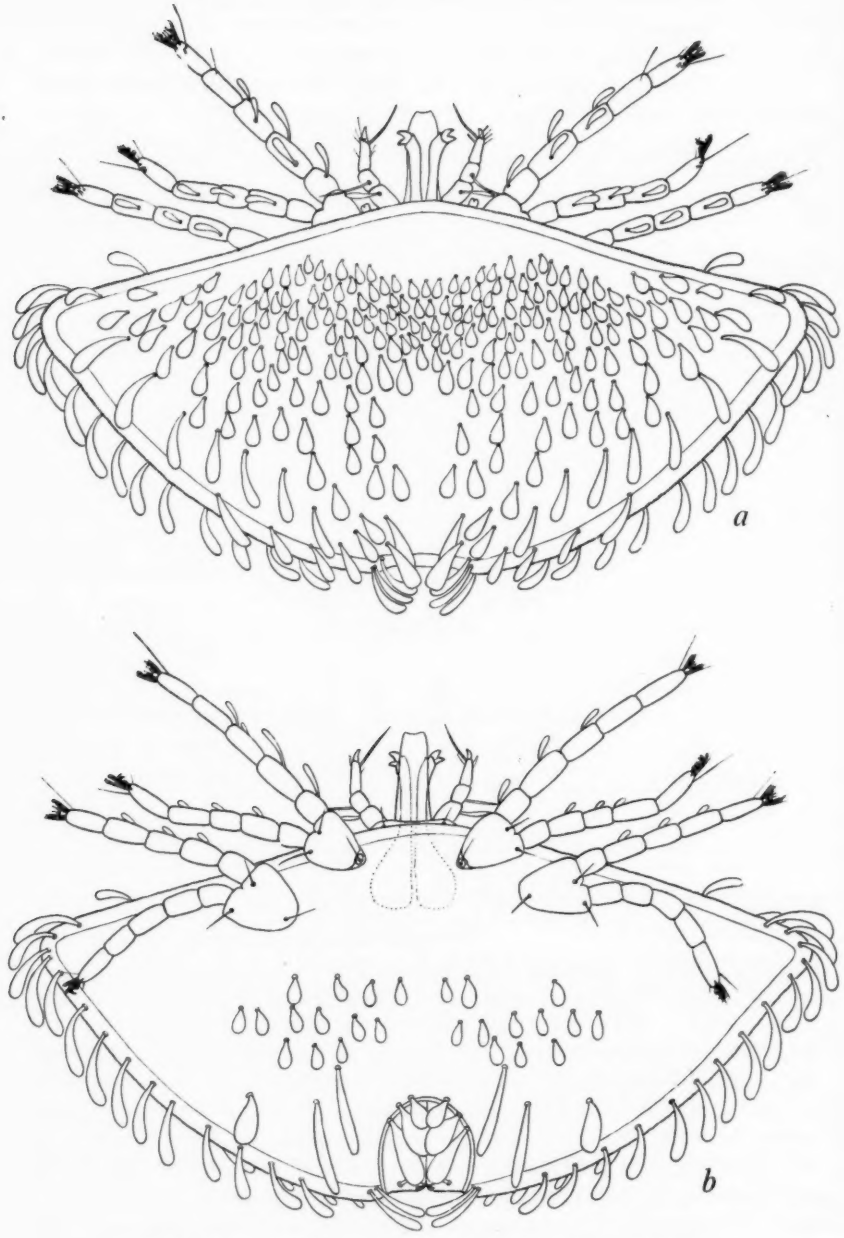


Fig. 6. *Zonurobia semilunaris*. Adult. *a*, dorsal view; *b*, ventral view.

their tips crossing, the middle pair considerably stouter than the remaining pairs; these spines are not shown in Fig. 6*b*, but in mounted specimens they can be seen through the transparent flattened hairs beneath which they lie.

Mouth-parts as in Fig. 6*a*, the mandibles and hypostome long and in spirit specimens usually projecting considerably further than is shown in this figure; segment II of palp with a more or less flattened hair above, segment III with a considerably longer slightly flattened hair with frayed edges, a setose spine medially below the claw not or very little stouter than the remaining spines of the segment; hypostome slightly notched apically.

Legs. Anterior common coxa with three spines, the proximal one situated on a slight basal projection of the chitin, posterior common coxa with three spines, the spine below its articulation with trochanter IV sometimes absent; remaining segments of legs as in Fig. 6.

Note. All flattened hairs with the usual fan-like striation; the sculpture of the anterior hairs of the dorsal surface (Fig. 1*f*) is strongly reminiscent of that seen in the shell of the common *Pecten* of the seashore.

Dimensions. Length 0·55 mm., breadth 1·13 mm.

Types. Twenty-three specimens from *Pseudocordylus microlepidotus*, locality of hosts unknown; other records from hosts taken at Bedford and Grahamstown. This species is found only on *P. microlepidotus* and its allies, the Grahamstown and Bedford hosts being perhaps better characterised as *P. fasciatus*. I have found these mites only on the tail scales, chiefly the ventral ones, to which they are loosely attached; their peculiar shape, the great breadth and reduced length, admirably adapts them to being concealed beneath the scale which is broad enough to cover them but whose free overlapping margin is only just deep enough to overlie them.

Zonurobia debilipes n.sp.

(Figs. 1*i*, 7)

Colour of spirit specimens light brown.

Body larger than in other species, considerably broader than long (Fig. 7*a*); the flattened hairs of the dorsal surface rather irregularly arranged, the peripheral hairs consisting of an irregular row of about fourteen large hairs inserted close to the margins of the dorsal surface and 7–10 similar ones inserted near the ventral margin; most of the dorsal surface occupied by about fifty hairs on each side these less than half the length of the largest peripheral hairs (Fig. 7*c*, *d*); anal region with a peripheral cluster of seven large hairs on each side, only one of these inserted dorsally, a pair of similar hairs above the apex of the anal field. Ventral surface with about twenty small hairs on each side of its posterior two-thirds; anal field as in Fig. 7*b*, with a group of 5–6 peripheral hairs and an oblique row of five flattened hairs on each side of the anal opening, lying under these two pairs of spines, the posterior considerably stronger than the other pair.

Mouth-parts as in Fig. 1*i*, seen from below; segments of palp long and

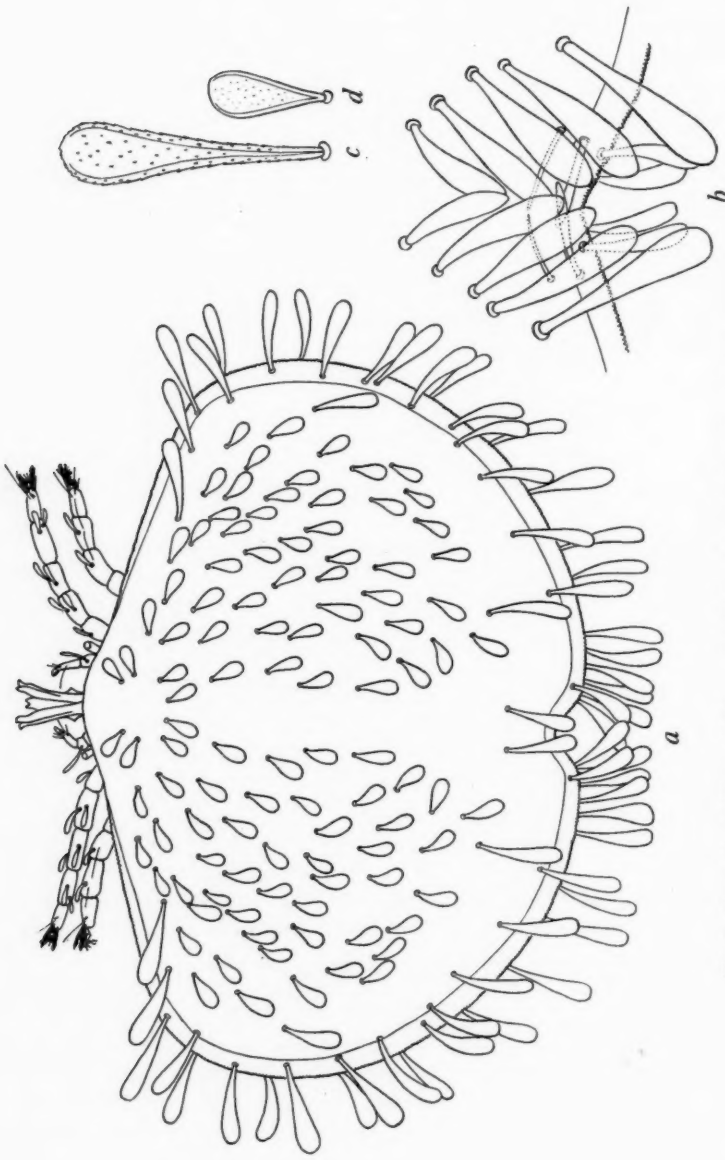


Fig. 7. *Zonurobia debilipes*. Adult. *a*, dorsal view; *b*, anal field, ventral view; *c*, peripheral hair enlarged; *d*, dorsal hair enlarged.

slender, segments II and III each with a flattened serrated hair above, that of segment II a little longer than that of segment III, segment IV long and slender armed with more or less equal sized spines, none of these very strong; hypostome notched apically.

Legs as in Fig. 7*a*, seen from above, small and weak as compared with the bulky body; anterior common coxa with two spines, one basal one and one below its articulation with leg I, posterior common coxa with only one basal spine; claws weak.

Note. The large peripheral hairs of this species are provided with a thickened lateral flange and armed along their whole length with sharp spicules (Fig. 7*c*).

Dimensions. Length 0·95 mm., breadth 1·3 mm.

Types. Fourteen specimens taken from five examples of *Zonurus warreni*, Ubombo, Zululand.

This mite occurs on no other species of *Zonurus*.

Zonurobia transvaalensis n.sp.

(Fig. 8)

Colour of spirit specimens light to dark brown.

Body in general appearance much resembling that of *debilipes* (Fig. 8); the peripheral hairs of outstanding size, their shape and sculpture as in *debilipes*, 14–17 large hairs implanted regularly along the dorsal surface, about the same number along the ventral surface these alternating with or overlapped by the dorsal peripheral hairs; dorsal surface with two rather unusually long pairs of hairs, the one above the anal field the other at about the middle. Ventral surface with about twenty-six hairs on each side; anal field with three pairs of small spines beneath the most posterior of five blunt tipped hairs which are inserted in a chitinous strip at the sides. *Mouth-parts* as in Fig. 8, differing from *debilipes* in having segments II and III each with a slender tapering cylindrical hair which is very faintly serrated; segment IV on the inner side below the claw with a long slender spine.

Legs as in Fig. 8.

Dimensions. Length 1 mm., breadth 1·26 mm.

Types in the Transvaal Museum, ten specimens from *Zonurus vandami*, Leydsdorp, Transvaal. Other specimens: fifty from *Z. vandami perkoensis*, East Transvaal; forty-two from *Z. barbertonensis depressus*, Louis Trichardt, Transvaal; four from *Z. laevigatus*, Entabeni, Zoutpansberg, Transvaal.

The mites of these mountain-frequenting Zonures from the Eastern Transvaal are obviously closely allied to those of *Z. warreni*, also a mountain-living lizard from Zululand; the above species differs from *debilipes* in the regularity of the dorsal and ventral peripheral hairs, the absence of a marked difference in size between the hairs of the periphery and the dorsal surface, and thirdly the structure of the hairs of segments II and III of the palp.

Zonurobia sanguinea n.sp.

(Fig. 9)

Colour in life dull red.

Body as in Fig. 9*a*, seen from above, longer than broad; periphery of body with a number of moderate hairs less regularly arranged than in other species, 8–10 inserted dorsally, about eight inserted ventrally; remaining hairs of dorsal surface 13–14 on each side; anal field situated dorsally with four blade-like hairs on each side inserted in a curved chitinous strip, a pair of hairs similar to

Fig. 8. *Zonurobia transvaalensis*. Adult, dorsal view.

the remaining hairs of dorsum immediately above the anal field and partly overlying it. Ventral surface in general appearance resembling the dorsal surface of other species, its posterior two-thirds fairly thickly covered with evenly spaced hairs these far exceeding those of the dorsal surface in number (at least thirty hairs on each side); anal region as in Fig. 9*b*.

Mouth-parts. Peritremes large and fully exposed; segment II of palp with a spine-like hair above not or very faintly serrated, segment III similarly with a long simple spine stouter and longer than that of segment II, segment IV long and slender, on the inner side below the claw with a long curved spine about the same length and thickness as that of segment III; hypostome long.

Legs as in Fig. 9*a*, seen from above inserted almost on the anterior edge of the body, not so definitely on the ventral surface as in other species; seen from below common coxae close together or touching, anterior common coxa with three, posterior common coxa with two spines, the basal spine of the anterior common coxa very long.

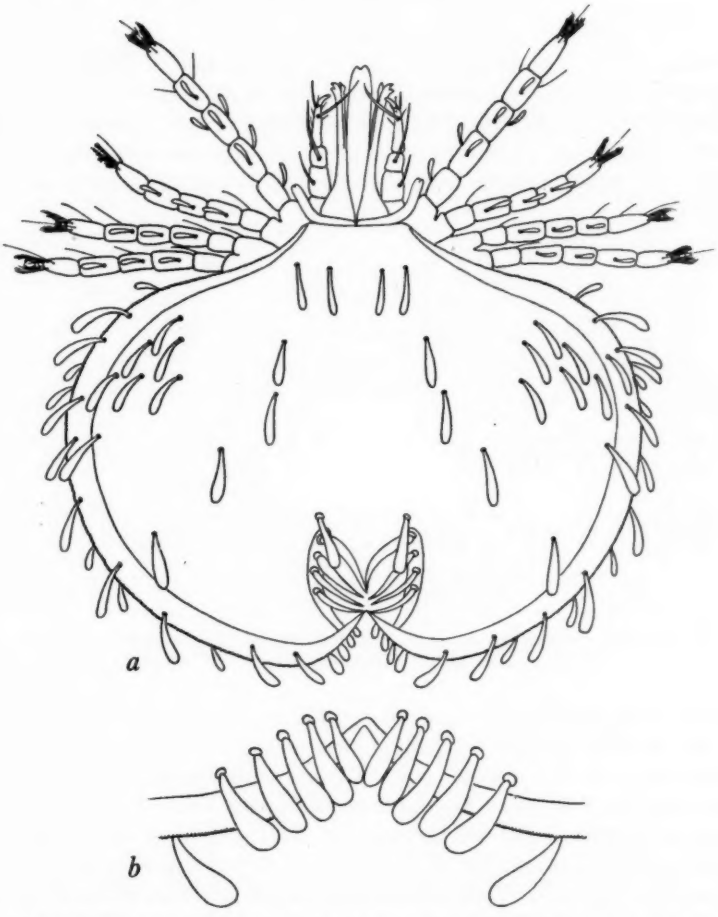


Fig. 9. *Zonurobia sanguinea*. Adult. *a*, dorsal view; *b*, anal field, ventral view.

Dimensions. Length 0·51 mm., breadth 0·74 mm.

Types. 100 specimens from *Zonurus coeruleopunctatus*, Montagu Pass, George. This species is peculiar in having (*a*) the anal opening with its associated spines and hairs situated on the dorsal surface, (*b*) the hairs of the ventral surface more numerous than those of the dorsal surface, (*c*) the mites being fixed

to the hosts in an inverted position, the ventral surface uppermost, the dorsal surface apposed to the body of the host and facing downwards; in respect of (*a*) and (*c*) the mite resembles *Ixodiderma inverta* (p. 24). They are attached chiefly to the sides of the belly and neck, the legs, arms, and occasionally the dorsal scales.

Zonurobia subquadrata n.sp.

(Fig. 10)

Colour in life usually dull reddish brown, lighter in unfed specimens. A small species closely resembling *Z. sanguinea* in its general habitus.

Body shaped as in *sanguinea*, broader than long, the anterior shoulders usually subrectangular; dorsal surface with hairs arranged as in *sanguinea*, the number and position of the hairs the same; anal opening situated on dorsal

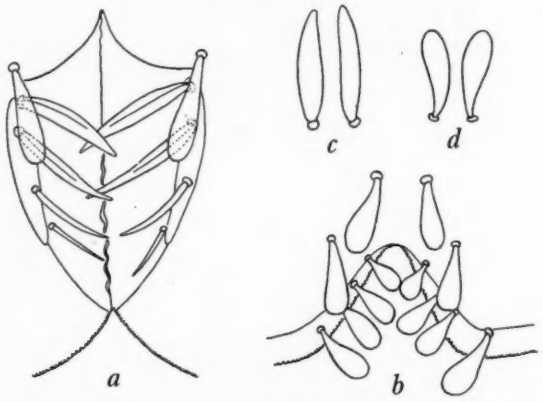


Fig. 10. *Zonurobia subquadrata*. Adult. *a*, anal field, dorsal view; *b*, anal field, ventral view; *c*, body hairs of ventral surface; *d*, the same of *Z. sanguinea*.

surface closely resembling that of *sanguinea* in its general appearance (Fig. 10*a*), the two posterior pairs of hairs being however spine-like and proportionately smaller than the two anterior pairs. Ventral surface as in *sanguinea* with a larger number of hairs than the dorsal surface (about thirty on each side) these being however blade-like and pointed (Fig. 10*c*) while those of *sanguinea* are shorter and club-shaped (Fig. 10*d*); ventral surface of anal field differing from *sanguinea* in having two pairs of hairs situated just above the middle (Fig. 10*b*).

Mouth-parts and *legs* as in *sanguinea*.

Dimensions. Length 0·53 mm., breadth 0·8 mm.

Types in the Transvaal Museum, 100 specimens from *Zonurus robertsi*, between Nieuwoudville and van Rhynsdorp. Other specimens, fifteen from the same host at Klaver, C.P.

This species differs from *sanguinea* only in the detailed structure of both the dorsal and ventral aspects of the anal region and in the shape of the hairs of

the ventral region; it resembles *sanguinea* in having the anal opening situated dorsally in contrast to all other species of *Zonurobia* where the reverse is the case; as in *sanguinea* it adheres to the host with its true ventral surface directed upwards and away from the surface of attachment.

Zonurobia montana n.sp.

(Fig. 11)

Colour of spirit specimens dirty white.

Body broader than long, dorsal surface as in Fig. 11*a*; periphery with 23–26 hairs truncated apically and sculptured as in *polyzonus* but much shorter than

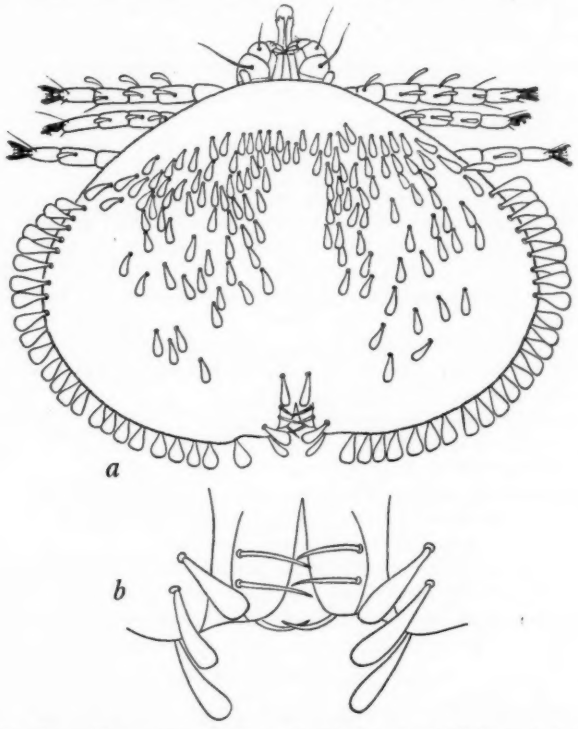


Fig. 11. *Zonurobia montana*. Adult. *a*, dorsal view; *b*, anal field, ventral view.

the peripheral hairs of this species, the anterior ones inserted dorsally, those near the anal field with a ventral insertion; dorsal surface with fairly numerous hairs smaller than the peripheral ones, anal region dorsally armed as in Fig. 11*a*. Ventral surface without hairs except for one a little above the anterior level of the anal field between it and the lateral margin, one a little above this hair, a third spine-like hair situated mesially to the last-named hair; anal field ventrally as in Fig. 11*b*.

Mouth-parts very stout, the whole forming a compact subtriangular structure as in *polyzonus*, the segments of the palp very short and stout, II and III above with a narrow cylindrical unserrated hair, segment IV with a large incrassate curved claw armed with a stout spine rising immediately from its base dorsally, swollen in its proximal half but tapering suddenly and curved in its distal half, the remaining spines of the segment very much slenderer; hypostome evenly rounded at its tip.

Legs as in Fig. 11*a*, seen from above; anterior common coxa with three, posterior common coxa with two spines, both common coxae roughly quadrate.

Dimensions. Length 0·65 mm., breadth 0·8 mm.

Types in Transvaal Museum, ten specimens from *Zonurus namaquensis*, Karasberg Mountains, South-west Africa.

This species resembles *polyzonus* in the shape and sculpture of the peripheral hairs, the general structure of the mouth-parts and anal region, but differs therefrom in its smaller size.

Key to the species of Zonurobia

1. Body almost circular, hairs of dorsum narrowing apically ... ... **circularis**
   Body considerably broader than long, hairs of dorsum widening apically 2
2. Body twice as broad as long, hairs of dorsum increasing progressively in size posteriorly ... ... **semilunaris**
   Body less than twice as broad as long, hairs of dorsum not increasing progressively in size posteriorly 3
3. Anal field situated dorsally, hairs more numerous on ventral than on dorsal surface 4
   Anal field situated ventrally, hairs more numerous on dorsal than on ventral surface 5
4. Hairs of ventral surface narrowing apically ... ... **subquadrata**
   Hairs of ventral surface widening apically ... ... **sanguinea**
5. Periphery of body with 12–17 hairs ... ... **cordylensis**
   Periphery of body with 20–30 hairs 6
6. Peripheral hairs long, anal region with five pairs of stout hairs 7
   Peripheral hairs shorter, anal region without five pairs of stout hairs 8
7. Dorsal hairs distinctly shorter than peripheral hairs ... ... **debilipes**
   Dorsal hairs very little shorter than peripheral hairs ... ... **transvaalensis**
8. Peripheral hairs all implanted dorsally, size large ... ... **polyzonensis**
   Only the anterior peripheral hairs implanted dorsally, size small ... **montana**

(2) *Scaphothrix* N.GEN.

Body considerably broader than long, a median sagittal section of a distended specimen almost circular, the boundary between the dorsal and ventral surfaces difficult to define; anal region situated at posterior apex of body, parts of it extending to both dorsal and ventral surfaces, the greater part of it visible from above; skin soft and delicate; hairs few in number, not more than seven pairs on either dorsal or ventral surfaces excluding the anal region, these hairs regularly though not strongly expanded distally, little flattened, with short spicules. Mouth-parts weakly developed and ill defined; palp always invisible in adult, infolded and appressed to the anterior margin of the body; hypostome not parallel-sided in its distal two-thirds, enlarging suddenly apically where it

is subtriangular seen from above, its sides curving round the mandibles to form an almost closed collar which does not quite meet dorsally; with the mandibles retracted the hypostome seen from directly above its long axis is almost circular and expanded into what appears to be an adhesive organ. Peritremes of stigmata indistinct and smaller than in *Zonurobia*. Legs spined as in other genera. Nymph and larval stages unknown.

Type and genotype, *Scaphothrix convexa*.

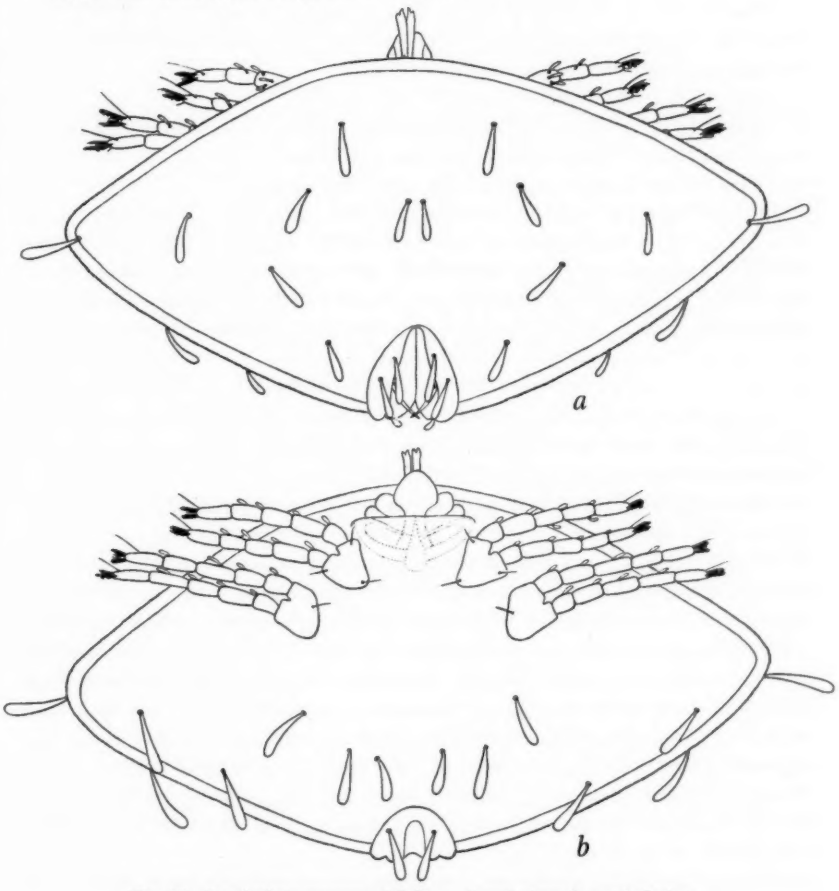


Fig. 12. *Scaphothrix convexa*. Adult. *a*, dorsal view; *b*, ventral view.

Scaphothrix convexa n.sp.

(Fig. 12)

Colour in life reddish, spirit specimens dull white to brown.

Body shaped as in Fig. 12*a*, seen from above; it is fairly constant being always convex both anteriorly and posteriorly but more so posteriorly; hairs

on dorsal surface arranged as in Fig. 12*a*, the anterior hairs with a fringe of spicules along their whole length, the posterior hairs smoother basally but with coarse spicules apically; ventral surface as in Fig. 12*b*; anal field as in Figs. 12*a*, *b*, the dorsal figure continuous with the ventral one; there are thus three pairs of stout hairs bordering the sides of the anal field; mesially to the second pair of hairs is a pair of large stout sharply pointed spines partly covered by these lateral anal hairs.

Mouth-parts as in generic description; though the segments of the palp cannot be clearly discerned they are evidently armed as two slightly flattened and serrated hairs project from this region.

Legs. Coxae and legs spined as in Fig. 12*a*.

I take these specimens to be adult as in several examples large eggs can be seen through the transparent skin of the abdomen.

Dimensions. Length 0·52 mm., breadth 0·95 mm.

Types. Twenty specimens from *Zonurus polyzonus*, Vondeling, Cape Province. Other records: from the same host, Kimberley, from *Z. peersi*, Garies (Namaqualand), from both black and brown varieties of *Z. cordylus*, Signal Hill, Cape Town. This species occurs together with *Zonurobia polyzonensis* on the same individuals of both *Zonurus polyzonus* and *Z. peersi*.

(3) *Ixodiderma* n.gen.

Adult. Body large, a little longer than wide, subparallel; skin leathery and blackish as in most Ixodidae, with fine corrugations; body usually provided with distally flattened hairs; anal field situated on dorsal surface, characterised by the usual folds and hairs; eyes very small, situated near the antero-lateral apices of the dorsal surface in association with a flattened hair.

Mouth-parts inserted in an invagination of the anterior margin of the body between the lateral shoulders, and in a vertical plane between dorsal and ventral transverse folds of cuticle of which the dorsal is the more anteriorly situated; palps (Fig. 13*b*) nearly always invisible or very indistinct, infolded and appressed to the sides of the body or hidden by the remaining structures of the mouth-parts, weakly developed; mandibles normal; hypostome with sides raised to form a conduit, curving round the mandibles and almost meeting above in the middle line, its apex differing from those of most other genera of Pterygosomidae in being greatly enlarged, forming a fleshy collar-like pad round the apices of the mandibles, the armed portions of which project from the middle of the fleshy ring.

Legs with coxae inserted near the anterior edge of the body, close to the mouth-parts and close to each other, slightly nearer to the ventral than to the dorsal surface, armed as in other genera with spines and hairs.

Nymph considerably smaller than adult, proportionally broader and more round; skin not as leathery as in adult but nevertheless resembling that of an Ixodid rather than the adult forms of other Pterygosomidae.

Mouth-parts peculiarly modified, being almost entirely covered ventrally by

a thin transparent chitinous scute which is cleft anteriorly in the middle line (Fig. 16); palps clearly visible, not infolded, segment III with a peculiar hyaline claw structure (Fig. 15*b*), apical segment nodular, clawless, and thickly spined, the whole palp resembling those of larval forms of certain species of Trombidiidae, *e.g. Thrombicula agamae* described by André (1929, Fig. 2). Eyes larger than in adult situated on a smooth plaque which includes a feathered hair. Hairs of both surfaces and legs as in adult but arranged differently.

Type and genotype. Ixodiderma inverta.

Remarks on the genus Ixodiderma

Perhaps the most striking feature of this genus, is the fact that in both the adult and nymph, the position on the host has become reversed; that is, the dorsal surface has become the ventral and *vice versa*, and therefore the dorsal side lies in contact with the body of the host. Presumably feeding and the general activities of the parasite take place in this position.

The mite, when removed from the host, presented an apparently normal aspect. The uppermost side was convex, polished in appearance with no anal opening; the underside was concave, unpolished, with a rather softer integument, which tended to become wrinkled and folded in spirit. However, on the closer examination of numerous mounted and unmounted specimens, a peculiar condition was found with relation to the apparent position of the mouth-parts. In all cases the hypostome appeared to lie dorsally to the mandibles and curved round below them; furthermore the peritremes of the stigmata always emerged in a ventral position instead of dorsally as in all other genera of Prostigmata; finally the minute eyes were seen on what had been presumed to be the ventral surface. There seemed therefore no escape from the conclusion that a complete reorientation had taken place so that the mite was, as it were, living and feeding on its back, the dorsal surface having become modified into a ventral one, and *vice versa*. If this was assumed the mouth-parts once more took up their positions in a vertical plane, with firstly the epistome above, then the mandibles, beneath these the hypostome, with the sides curving upwards, and, in the case of the nymph, most ventrally of all a scute; the eyes then appeared on the dorsum, and the peritremes of the stigmata emerged dorsally, situations which, as far as I know, are normal in all other Prostigmata. Owing to the coxae being implanted almost on the edge of the anterior margin of the body, the position of the legs in the adult afford less aid in distinguishing the dorsal from the ventral surfaces, than would be inferred from the ventral position of these appendages in nearly all Acari; in the nymph, however, the large well-defined piece of chitin forming the exposed surfaces of the coxae with their characteristic spines are clearly visible on the ventral surface.

This large chitinous ventral plate (Figs. 15, 16) is in its form described in the nymph of *Ixodiderma*, a structure that has not been met with heretofore in Prostigmata; it appears to be quite distinct from the large hypostome com-

pletely overlying as it does both this and the other structures of the mouth. On the other hand this may be only apparent, as it is much less easy to follow the various organs and their insertions towards the base of the buccal cavity than is the case in *Zonurobia*. The plate may be in reality an extraordinary development of the thin chitinous membrane which overlies the coxae of the palps in *Zonurobia* (Fig. 1*i*). This thin transparent layer of chitin seems to cover the coxae ventrally while distally it fuses with the hypostome, the two forming a single piece which lies slightly more ventrally than the palps and maxillae, the last named being distinguishable here as two separate pieces at the base of the hypostome. The spines situated distally on the ventral plate of *Ixodiderma* (Fig. 16) can only be compared with those seen overlying the coxae at the base of the hypostome in *Zonurobia* (Fig. 1*i*), and if they are indeed homologous with these would indicate a great development of this coxal plate in an anterior direction, becoming detached from and overlapping the hypostome and pedipalps. It seems to be a temporary modification during the nymphal stage and may serve for the protection of the exposed and incompletely developed mouth-parts to prevent them from being damaged by movements of the host through grass or over stony and uneven ground; the microscopic striations on the apparent dorsal surface of the adult, obviously due to these agencies, is remarked on below in the detailed description of the genotype. In the adult there is no indication of this large plate.

Ixodiderma inverta n.sp.

(Figs. 13, 14, 15, 16)

Adult. Colour. Spirit specimens blackish brown, the dorsal surface (the apparent ventral surface) dull, the ventral surface shiny.

Dorsal surface as in Fig. 13*a*, the dorsal and peripheral hairs enlarged and flattened distally with striations and sculpture in their distal halves; the minute eye situated anterior and mesially to a large anteriorly placed hair on the lateral shoulder; eye several diameters from its associated hair and very little larger than the ring surrounding the base of this hair; anal field as in Fig. 13*a*, the actual anal opening with a transverse fold of more strongly chitinised skin on each side ending in a blade-like point.

Mouth-parts as in generic description; the relations of the various structures to each other difficult to define owing to the great enlargement of the hypostome apex which obscures the remaining parts of the mouth; the epistome is a thin transparent piece of chitin, rectangular and considerably broader than long; beneath the epistome the hypostome is the most noticeable structure enclosing the mandibles, only the armed tips of which can be seen projecting from its swollen knob-like apex; the palps rather small and as in Fig. 13*a*, so closely compressed that the individual segments cannot be distinguished; when teased out they appear as in Fig. 13*b*, with a distinct claw on the terminal segment and the usual hairs and spines.

Legs with coxae inserted close to the anterior edge of the body, their ex-

posed surfaces visible on the ventral surface; common coxa of legs I and II with three extremely long spines, one basal spine and one below each articulation with the legs, common coxa of legs III and IV with apparently only one smaller basal spine; remaining segments of legs with rather slender flattened hairs.

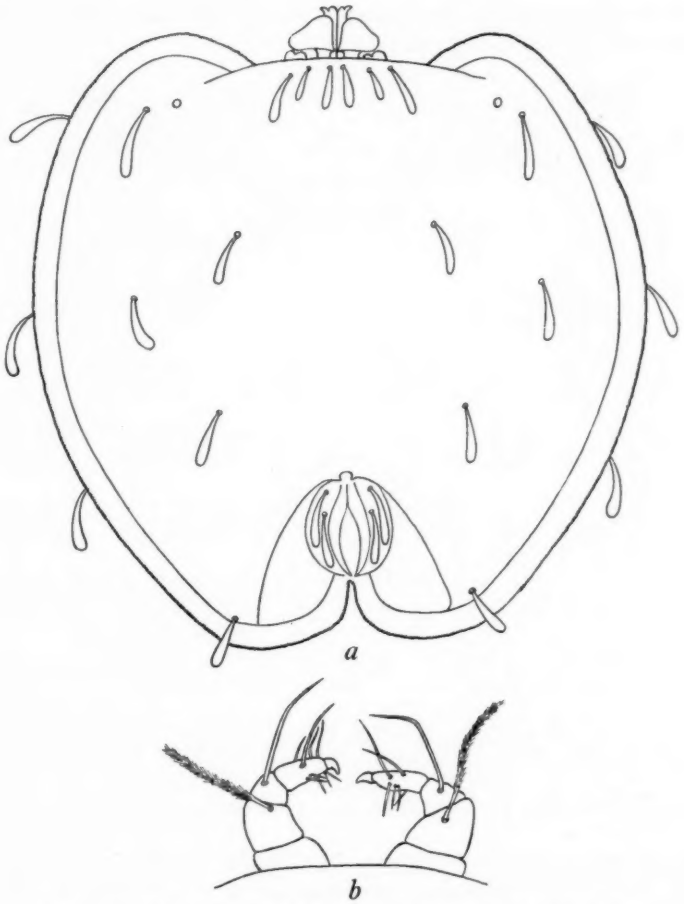


Fig. 13. *Ixodiderma inverta*. Adult. *a*, dorsal view (the legs not shown); *b*, the palpi, enlarged.

Nymph as in Figs. 15, 16, more rounded than in adult; the ventral scute consists of an apparently complete piece of chitin with a thickened rim posteriorly and at the sides; it is thin and so transparent, especially anteriorly, that when looked at from the ventral side all the structures of the mouth-parts can be seen through it; the shape is roughly quadrate and shovel-like; the

anterior hood is provided with an oblique incision so that the one half of the hood overlies a small portion of the other half, each flap being provided with a small spine, in addition a small transverse cut about the level of the bases of the flaps can be seen in some specimens; the scute covers parts of the anterior common coxa at the sides and all of the mouth-parts except a small anterior part of the hypostome and mandibles; eyes as in Fig. 15*a*, a little larger than in adult, anal field as in Figs. 15*a,c*.

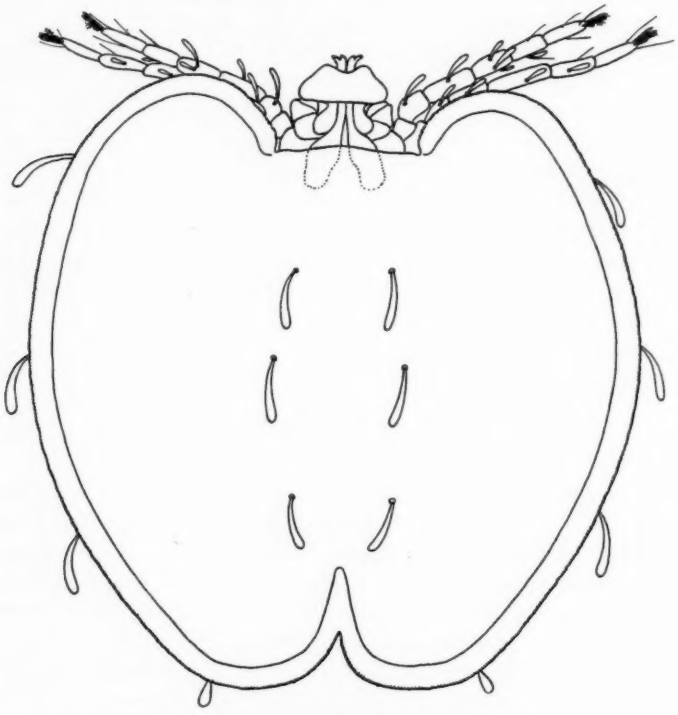


Fig. 14. *Izodiderma inverta*. Adult, ventral view.

Mouth-parts seen from above as in Figs. 15*a,b*; the following structures are seen in the order in which they occur from above downwards: most dorsally the broadly triangular epistome, its basal angles overlying a small part of the anterior common coxa; just beneath these the peritremes of the stigmata emerge at a point where the epistome meets the common coxa as it overlies it, the peritremes thus emerge between the epistome and common coxa in a vertical plane and a little anterior to the common coxa in a horizontal plane; below the epistome are the mandibles which in Fig. 15*b*, have been drawn aside to show the hypostome with the palps inserted near its base; the apex of the hypostome is fleshy and thickened and its structure is granular in contrast to the

transparence of the rest of the segment; the segments of the palp are much more swollen than in the adult with hairs and spines as in Fig. 15*b*, penultimate segment with a sinuous hyaline structure inserted near its ventral distal apex, this structure slightly expanded at its tip which is provided with 5–6 small lobules

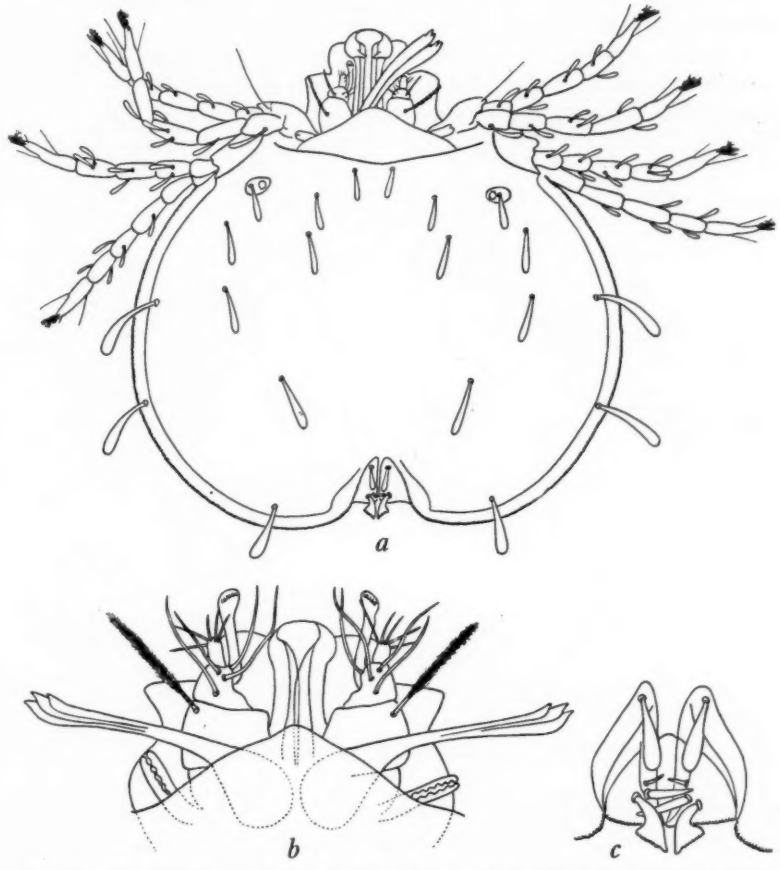


Fig. 15. *Ixodiderma inverta*. Nymph. *a*, dorsal view; *b*, mouth-parts of same enlarged; *c*, anal field of same enlarged.

giving it the appearance of a little clenched fist; though unlike a claw in appearance this structure undoubtedly corresponds to the claw on the same segment of the palp in larval Trombidiidae; last segment of palp small, cylindrical, with numerous spines.

Legs. Anterior common coxa large, its free surface fully exposed ventrally (Fig. 16), a long spine on its dorsal anterior margin near the insertion of leg I, in addition two ventral spines; posterior common coxa smaller, longer than

broad, with one ventral spine; remaining segments of legs with flattened, feathery hairs.

Dimensions. Adult: length 1·3 mm., breadth 1·2 mm.; nymph: length 1 mm., breadth 0·9 mm.

Types. Twenty-seven adults and nymphs from *Pseudocordylus subviridis*, Belfast, Transvaal.

Numerous specimens of adults and nymphs from the following records:

From *P. microlepidotus*: Table Mountain and two unknown localities. *P. fasciatus*: Katberg, Coetzeesberg, Bedford, Butterworth, Grahamstown, Somerset East (Cape Province). *P. subviridis*: Leydenburg, Woodbush,

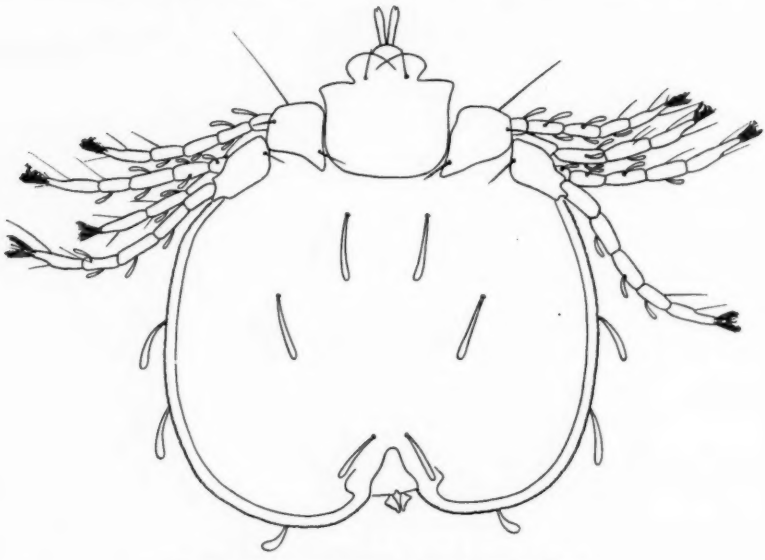


Fig. 16. *Ixodiderma inverta.* Nymph, ventral view.

Ermelo, Carolina, Wakkerstroom (Transvaal), Thaba Putsua, Maletsunyane Falls (Basutoland), Giants Castle (Natal). *Zonurus coeruleopunctatus*: Montagu Pass, George. *Z. polyzonus*: Steinkopf, Lamberts Bay, Phillipstown, Cradock (Cape Province); Aus, Krailuft (South-west Africa).

Note on Ixoderma inverta

Unmounted adult specimens vary in shape being more rounded in fully fed individuals; in distended specimens the angle formed by the posterior cleft of the anal region may be more or less obliterated. Under low power of the microscope the shiny dorsal surface (*i.e.* the true ventral surface) shows fine but distinct grooves and scratches, most of these grooves being longitudinal but a few oblique or transverse; it seems most probable that this is due to

friction caused by the body of the host coming into contact with the ground or moving through grass or other surrounding objects. The general appearance of adult rather resembles that of an immature tick.

Ixodiderma lacertae n.sp.

(Fig. 17)

Colour as in preceding species.

Adult as in Fig. 17, seen from above; differing from *inverta* in the mouth-parts being sunk in a very deep invagination of the anterior margin of the body,

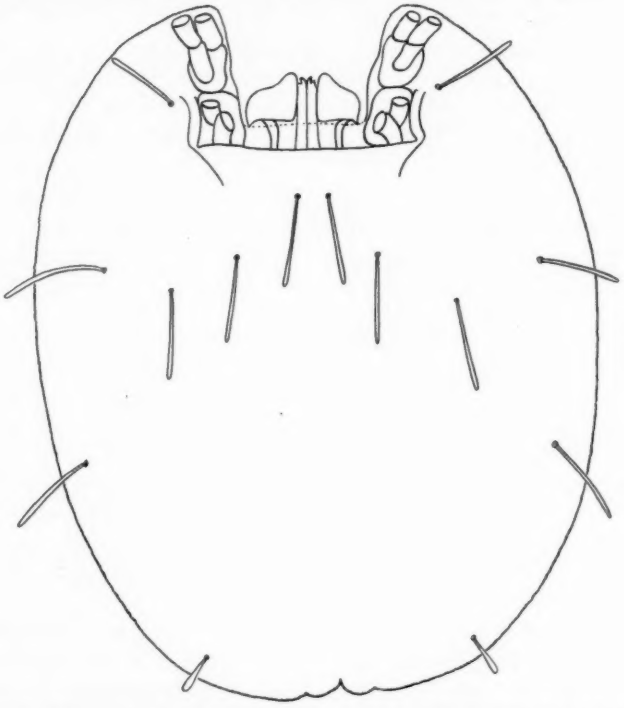


Fig. 17. *Ixodiderma lacertae.* Adult, dorsal view (distal segments of the legs not shown).

the lateral shoulders prominent and projecting far beyond the anterior extremity of the mouth-parts; the mouth-parts themselves, especially the hypostome, broader than in *inverta*; the dorsal surface with hairs arranged as in Fig. 17, the hairs themselves differing from those of *inverta* in being slenderer and showing little signs of apical flattening, provided with minute spicules. The hairs of the ventral surface and anal field cannot be made out.

Legs definitely situated nearer to the dorsal than the ventral surface, the coxae inserted as in Fig. 17, close to the mouth-parts along the mesial margin

of the shoulders; their position is so much modified by the deeply sunk mouth-parts that the common coxa of legs III and IV is here situated anteriorly to that of legs I and II.

Nymph differing from that of *inverta* in the following details: hairs of body much longer, cylindrical, dorsal surface with ten equal-sized hairs on each side, anal field with two large hairs on each side, ventral surface with three pairs of hairs in the middle; eye well separated from the smooth plaque containing its associated hair; mouth-parts without a ventral scute; legs with cylindrical hairs much longer than those of *inverta*.

Dimensions. Adult: length 1·3 mm., breadth 1·1 mm.; nymph: length 0·66 mm., breadth 0·74 mm.

Types. Six adults, four nymphs from *Scapteira depressa*, locality of host unknown.

Other records: Two adults from *Scapteira knoxii*. One adult, three nymphs from *Scapteira suborbitalis*. Four adults from *Tropidosaura gularis*. One adult, one nymph from *Eremias undata*. (Localities of hosts of above unknown.) Four adults from *Eremias undata*, Luberitzbucht, South-west Africa. Six adults from *Eremias inornata*, Narudas Sud, South-west Africa.

It will be noticed that this species occurs only on lizards of the family Lacertidae.

Ixoderma pilosa n.sp.

(Fig. 18)

Colour. Spirit specimens dull blackish brown.

Adult. Body almost round about as long as broad (Fig. 18*a*), its dorsal surface with long cylindrical hairs, these of more or less even thickness, serrated, not tapering apically (Fig. 18*b*); eyes well separated from their associated hairs; anal region situated at posterior apex of abdomen for the most part on the ventral surface, its dorsal portion armed as in Fig. 18*a*. Ventral surface with hairs similar to those of the dorsal surface, about thirty occupying a roughly quadrate area in the middle of the body broader than long, in addition some hairs at the lateral margins of the body and a pair of considerably smaller hairs in the middle just posterior to the mouth-parts; anal region seen from below as in Fig. 18*c*, a row of 6–7 hairs at the sides, these a very little stouter than the remaining hairs of the body.

Legs as in Fig. 18*a*, clothed with unusually long slender hairs resembling those of the body but shorter and more slender.

Dimensions. Length 1·54 mm., breadth 1·54 mm.

Types. Four adults from *Pseudocordylus subviridis*, Drakensberg, Basutoland; other specimens, four adults from the same host, Mont-aux-Sources, Basutoland. Twenty nymphs from the same host as the types do not seem to differ in any way from those of *Ixodiderma inverta* and probably do not belong to the above species.

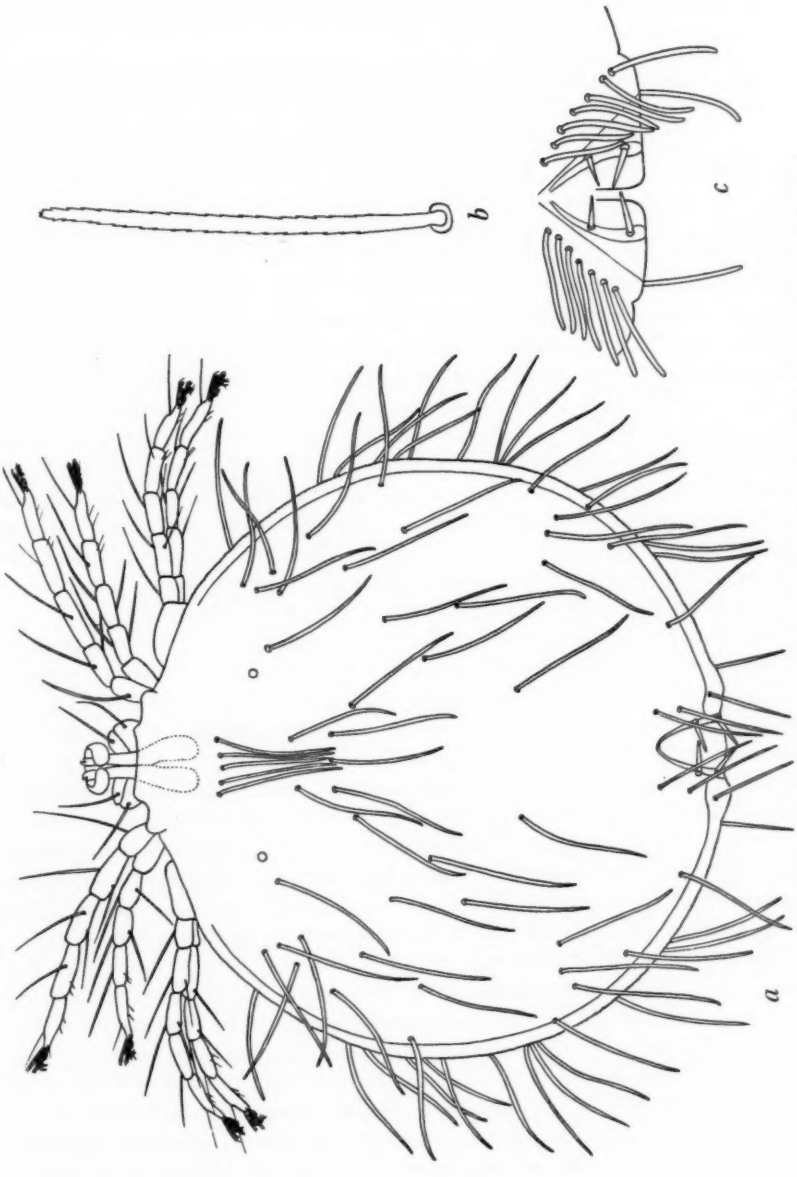


Fig. 18. *Ixodiderma pilosa*. Adult. *a*, dorsal view; *b*, a body hair enlarged; *c*, anal field, ventral view.

Key to species of Ixodiderma

1. Dorsal surface with numerous long cylindrical hairs ... ... **pilosa**
   Dorsal surface with fewer and shorter hairs 2
2. Anterior sides of body projecting far beyond the mouth-parts ... **lacertae**
   Anterior sides of body not projecting beyond the mouth-parts ... **inverta**

The mechanism of attachment in Ixodiderma

This genus differs from other Prostigmata parasitic on lizards in that it is far more securely attached to its host; as in the case of Ixodidae it is often difficult to detach the mite without injuring or breaking off the mouth-parts and leaving these embedded in the skin of the host. In most cases when removing the mite from the body of the host a small piece of skin comes away with it, which when examined under the microscope, reveals the fact that the hypostome with the enclosed mandibles has penetrated the skin, and retraction being effectually prevented by the swollen fleshy apex, the skin is carried round it like a ruff or collar. It is difficult to understand how the large fleshy apex of the hypostome can have passed through the much smaller aperture in the skin; it would seem obvious that the enlargement takes place after the hypostome has passed through the puncture made by the mandibles; this would predicate a certain power of turgescence in the apical portion of the hypostome brought about perhaps by the pressure of saliva; this seems to be supported by the fact that in one or two of the nymphs the hypostome is normal and subparallel, the apex not being swollen or only to a slight extent. The fleshy sucker-like appearance of the hypostome may therefore be a temporary condition appearing in specimens which have undergone more or less prolonged fixation to the host. The swollen apex of the hypostome differs also in its finer structure from the remaining transparent proximal portion, having a granular or alveolar appearance which may be due to small bubbles of air or liquid. In other genera in which the hypostome is not enlarged apically, such as *Zonurobia* and *Pterygosoma*, there is no differentiation in the structure of the organ, the whole hypostome being transparent and homogeneous throughout. Trouessart (1897) describes in the case of a species of *Trombidium* an adventitious tube or vesicle formed round the hypopharynx (*tube suceur, langue*) in the subcutaneous tissue of a mammalian host, the tip of which recalls in appearance the swollen hypostome apex in *Ixodiderma*, and he thinks this tube is formed by the coagulatory action of the saliva of the mite on the blood serum of the host. In the case of *Ixodiderma*, the size of the hypostome after the removal of adventitious material is not appreciably diminished. This cutaneous material, derived from the tissues of the host, can be removed by means of KOH or oil of creosote. It seems therefore that the enlargement of the hypostome in adults and nymphs is a structural part of the hypostome itself and it certainly resembles the collar-like hypostome of certain larval *Allothrombium* species described by Oudemans (1909). It is evident that this modification has the same function as the recurved teeth of the hypostome of Ixodidae, namely

assisting the animal in retaining a firm hold on the body of its host over prolonged periods of time.

IV. MITES PARASITIC ON GERRHOSAURIDAE

(4) *Pterygosoma* Peters

Pterygosoma gerrhosauri n.sp.

(Fig. 19)

Colour in life dull red, spirit specimens light yellow to brown.

Body as in Fig. 19*a*, seen from above; hairs of the shoulders numerous forming a compact group, cylindrical, moderately long, with weak serrations

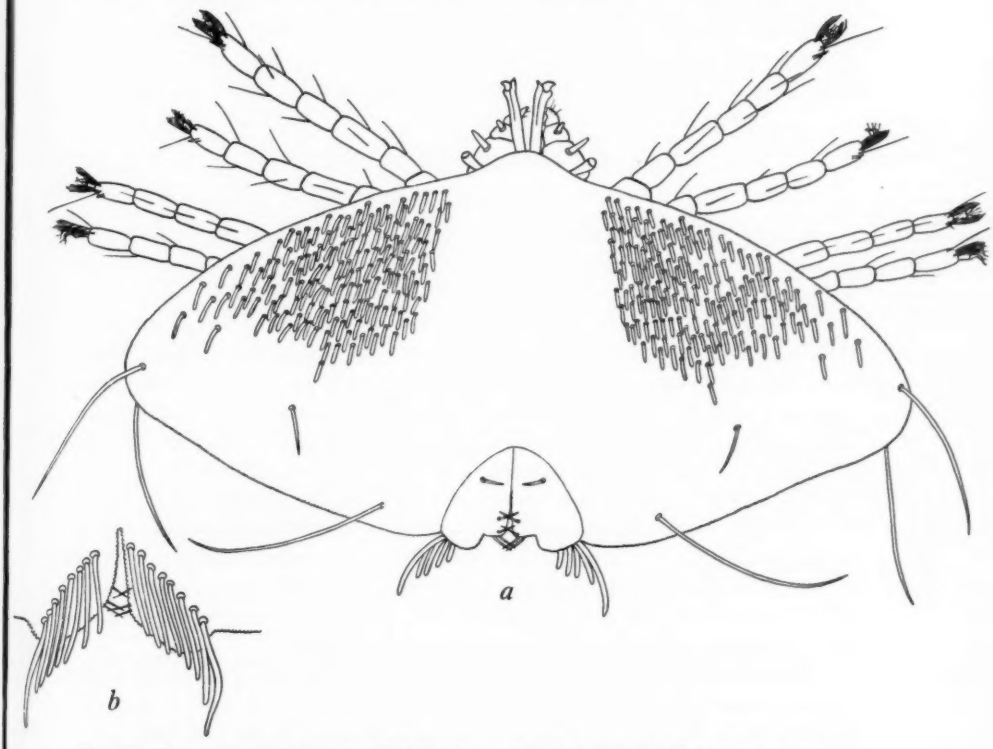


Fig. 19. *Pterygosoma gerrhosauri*. Adult female. *a*, dorsal view; *b*, anal field, ventral view.

along their whole length, their apices truncate; peripheral hairs three on each side, long and slender, two at each lateral apex and one posteriorly and more mesially, the latter inserted on the ventral surface the two others on the dorsal surface; one pair of intermediate hairs on the dorsal surface. Ventral surface with only one pair of small spines, these placed laterally to the upper level of

the anal field; anal field as in Fig. 19*b*, seen from below, an oblique row of about eight strong blunt tipped spines on each side, some much smaller spines beneath and between them.

Mouth-parts normal as in Fig. 19*a*; segment II of palp with a blunt conical hair above provided with very faint serrations, segment III with a very low blunt cone above, segment IV with a strong spine below the claw, stout basally but tapering suddenly in its distal half.

Legs. Anterior common coxa with three spines the basal one well separated from the chitinous portion of the coxa, posterior common coxa with two spines, one basal and one near its articulation with leg III; remaining segments of legs with slender spines, none of them serrated.

Dimensions. Length 0·8 mm., breadth 0·4 mm.

Types. Twelve specimens from *Gerrhosaurus validus*, Zululand. Other records: four specimens from the same host, Plumtree, Rhodesia: two specimens from *Gerrhosaurus major grandis*, Leydsdorp, Transvaal.

Pterygosoma bicolor n.sp.

(Figs. 20, 22*c*)

Female. Colour of spirit specimens white, the intestinal caeca giving the central parts of the body a black colour contrasting strongly with the periphery.

Body as in Fig. 20*a*, seen from above, the number of peripheral hairs larger than in *P. gerrhosauri*; anterior hairs of the dorsal surface differing strongly from the more posterior ones in size and structure being short cylindrical and truncated apically where there is also a small cleft or notch; these hairs almost smooth with very faint serrations, the peripheral hairs long and slender, tapering to a fine point, completely smooth; a large number of hairs intermediate in size between these two types; ventral surface with only two hairs on each side resembling the long peripheral hairs of the upper surface, one on each side of the upper level of the anal field and one about halfway between this and the lateral apex; anal field below, as in Fig. 20*b*, with an oblique row of 5–7 (usually six) stout spines on each side, between and beneath these three pairs of smaller spines (sometimes only one or two pairs).

Mouth-parts as in Fig. 20*c*, seen from above; segment II of palp with a long slender dorsal spine weakly serrated apically, segment III with a stout triangular spine, segment IV with a stout spine mesially below the claw.

Legs. Anterior common coxa with three spines, the basal one more or less separated from the coxa, posterior common coxa with two spines, one basal and one below its articulation with leg III; all legs with very slender spine-like hairs, those of the second segment of all legs larger than the others and very weakly serrated.

Dimensions. Length 0·44 mm., breadth 0·9 mm.

Male. Body as in Fig. 22*c*, seen from above, with two long lateral spines

the anterior one inserted dorsally the posterior ventrally; ventral surface unarmed except for two small spines on each side, one above the anal cleft and one between this and the posterior lateral spine; anal region itself unspined; penis indistinct, of the same general form as that of *P. hystrix* (Fig. 22*b*), lying within a chitinous V-shaped structure; mouth-parts and legs as in Fig. 22*c*, spination of coxae as in female.

Dimensions. Length 0·27 mm., breadth 0·4 mm.

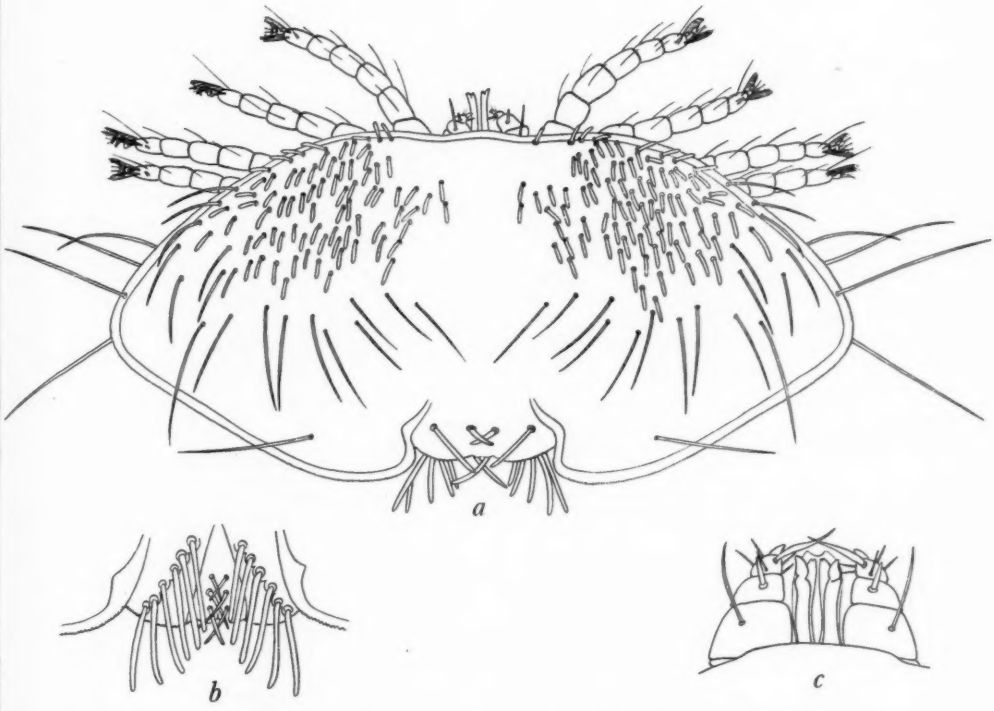


Fig. 20. *Pterygosoma bicolor.* Adult female. *a*, dorsal view; *b*, anal field, ventral view; *c*, mouth-parts, dorsal view.

Types. Seventy specimens from *Gerrhosaurus flavigularis*, Serowe, Bechuanaland. Other records: from *Gerrhosaurus nigrolineatus*, locality unknown; *G. auritus* Kaokoveld, South-west Africa.

These mites occur only on the ventral scales of the belly, legs, arms and tail where they are quite covered by the scale; specimens predominantly black in colour are more or less visible through the scale. This species appears to be a form intermediate between *gerrhosauri* and *hystrix*.

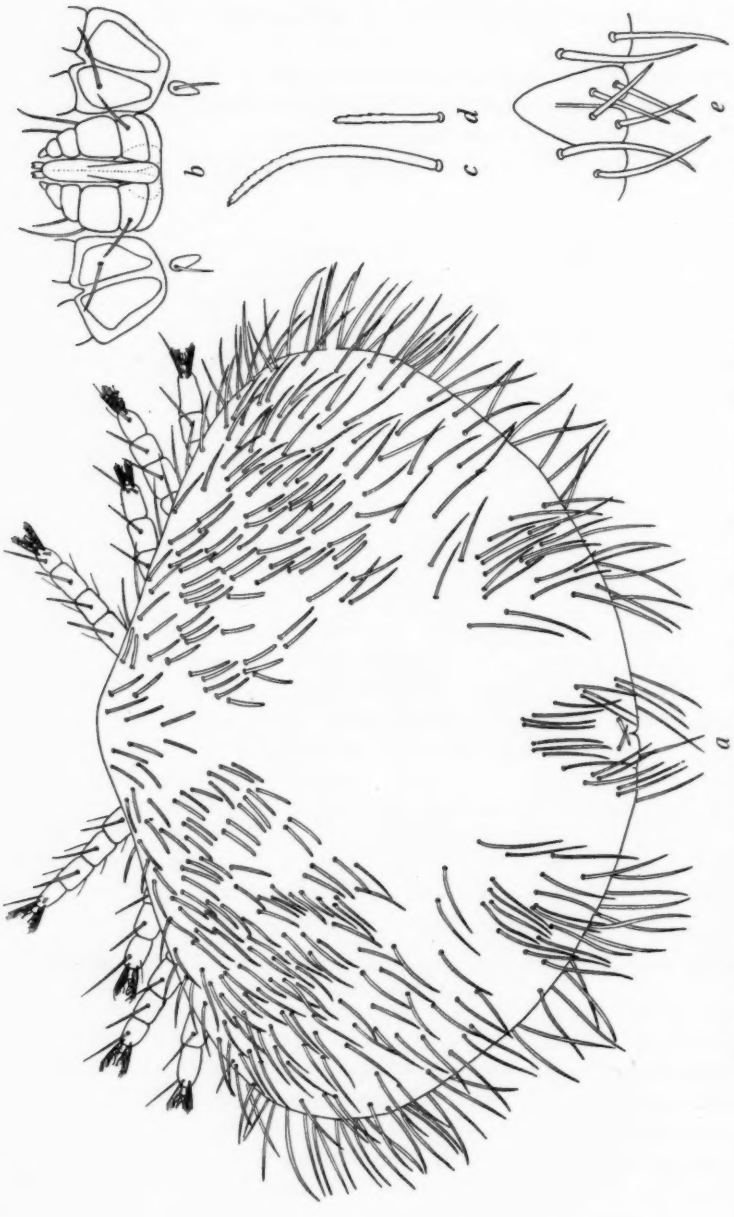


Fig. 21. *Pterygosoma hystrix.* Adult female. *a*, dorsal view; *b*, mouth-parts and first common coxa, ventral view; *c* and *d*, posterior and anterior dorsal hairs respectively, enlarged; *e*, anal field, ventral view.

Pterygosoma hystrix n.sp.

(Figs. 21, 22*a*,*b*)

Female. Colour of spirit specimens dull grey to blackish, a more or less variable central portion white, the black predominating over the white.

Dorsal surface as in Fig. 21*a*; body in fully fed adult specimens bulky, overlying the mouth-parts which are usually concealed from view; hairs numerous and rather irregular, the anterior considerably smaller than the

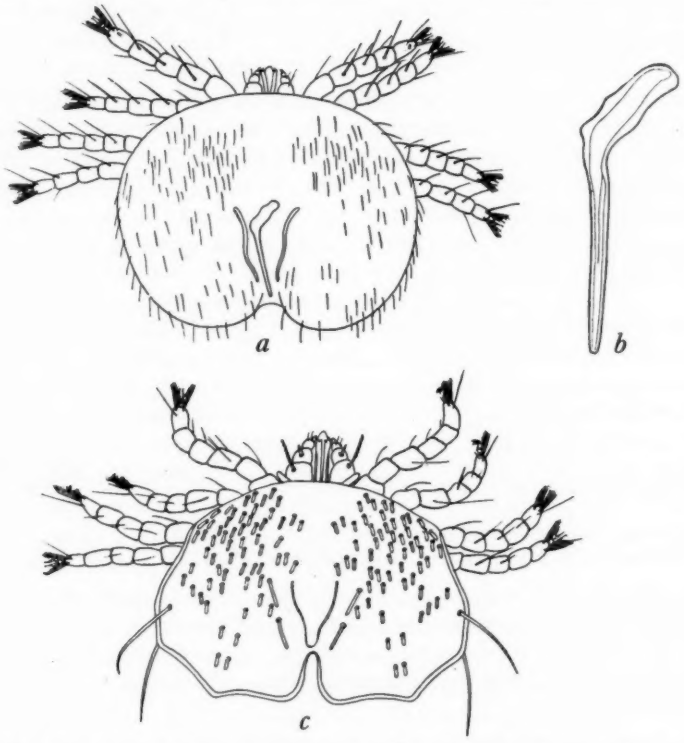


Fig. 22. *a*, male of *Pterygosoma hystrix*, dorsal view; *b*, penis of same, enlarged; *c*, male of *P. bicolor*, dorsal view.

posterior ones, terminating bluntly or with a slight cleft (Fig. 21*d*); no sudden transition between the short anterior and the long posterior hairs as in the preceding species, there being a number of intermediate types passing gradually into the long pointed hairs of the posterior margin (Fig. 21*c*); ventral surface with a considerable number of hairs (about fifty) of the long slender type, the great majority of these arranged in a group at the side, a few on each side of the anal field; anal field seen from below as in Fig. 21*e*.

Mouth-parts seen from below as in Fig. 21*b*, small in proportion to the body;

segments II and III of palp with a longish hair above, that of II a little longer than III, these hairs not or weakly serrated.

Legs as in Fig. 21*a*, coxa I spined as in Fig. 21*b*.

Dimensions. Length of body 1·1 mm., breadth 1·53 mm.

Male. Colour white.

Dorsal surface as in Fig. 22*a*, fairly thickly and irregularly covered with fine hairs, the posterior a little longer than the anterior ones, both anterior and posterior hairs truncate and very weakly serrated; ventral surface with a median pair of cylindrical hairs near the anal cleft posteriorly, a smaller pair halfway between the posterior margin and anterior common coxa; a group of about seven long cylindrical hairs at the postero-lateral apices of body and between these and the anal pair a similar hair. Penis seen through the skin as in Figs. 22*a*,*b*; in unmounted specimens it can be only vaguely discerned; mouth-parts as in female, coxae as in female, legs with numerous long hair-like spines; claws large.

Dimensions. Length of body 0·47 mm., breadth 0·6 mm.

Types. Fifty specimens from *Gerrhosaurus flavigularis*, Grahamstown. Found under the ventral scales of belly and tail.

Key to species of Pterygosoma *occurring on Gerrhosauridae*

1. Dorsum thickly and irregularly covered with hairs not differing greatly in size **hystrix**
   Dorsum with hairs confined to definite areas, the anterior differing markedly from the posterior hairs in size **2**
2. Dorsum with one pair of hairs intermediate between the anterior and posterior hairs in size ... ... **gerrhosauri**
   Dorsum with numerous hairs of intermediate size ... ... **bicolor**

V. GENERAL DISCUSSION

The Zonuridae with the exception of the genus *Chamaesaura* are parasitised by one or more of the three genera *Zonurobia*, *Scaphothrix* and *Ixodiderma*. There is no interchange of parasites between such rupicolous families, as for instance the Zonuridae and the Agamidae; if this were the case most favourable opportunities would occur in the Cape Peninsula, where in many localities *Zonurus cordylus* and *Agama atra* are found living together in the fissures of the same rocks. Both families occur throughout South Africa, and Zonuridae and Agamidae very probably live communally in many localities. *Pterygosoma* is the only scale mite found on South African Agamids and I have never found it living on a Zonurid, or *Zonurobia* on an Agamid. *Zonurobia* is a true scale parasite; usually it fixes itself with only the mouth-parts covered by the scale —*Z. cordylensis*, *polyzonensis*—or less frequently with much more of the body covered, so that the mite is almost concealed—*Z. semilunaris*.

Ixodiderma is not a true scale parasite, but with the aid of its peculiarly modified mouth-parts may be adherent on any part of the body of its host, as in the Ixodidae. It is very often attached to the fingers or toes of the host, but

if found on a flat surface such as the belly or flanks it fixes itself between and not under the scales. On account of its tough epidermis it can probably withstand far less favourable conditions than *Zonurobia*. The actual numbers found on individual hosts are usually fewer than in *Zonurobia* or *Scaphothrix*.

Scaphothrix occupies a position more or less intermediate between these two genera, possessing a soft skin but modified mouth-parts like those of *Ixodiderma*; it seems, however, definitely to fix itself under the scales of its host in much the same manner as *Zonurobia*.

These genera also differ from one another in that some of them are confined to a specific host, whereas others are more catholic in their distribution. In *Zonurobia*, for instance, most of the species may be said to have a specific host on which with one or two exceptions they are invariably found. On the other hand *Ixodiderma* is not limited to a single specific host, for *I. inverta* has been found on two species of *Zonurus* and on the various forms of *Pseudocordylus*, the genus which it most frequently adopts as host; *I. lacertae* occurs only on three Lacertid genera. The mouth-parts and robust exterior of *Ixodiderma* give the impression that it can exist on a fairly wide range of lizards, embracing more than one family. *Scaphothrix* cannot be said to be specific for any Zonurid lizard, being found on three species of the genus *Zonurus*.

The Gerrhosauridae. Members of this family are parasitised by one genus of prostigmatic mites, though in certain cases cited on p. 2 they are also the hosts of Mesostigmata. It would be difficult to explain on the ground of the phylogenetic affinities of the hosts why the Gerrhosauridae should be parasitised by the genus *Pterygosoma*, which is also the only genus found on the South African members of Agamid lizards. The Gerrhosauridae are considered as forming together with the Scincidae and Lacertidae a distinct group or section; in their characters they are perhaps intermediate between these two families; they certainly have no relationships with the Agamidae. The three species of *Pterygosoma* found on the genus *Gerrhosaurus* could be said to form a group distinct from those species found on Agamidae, and might be separated under a subgenus; but nevertheless there is no doubt that they are much more closely related to *Pterygosoma* than to any other prostigmatic genus found on lizards.

(*a*) *Mite parasitism in relation to lepidosis*

The absence of mite parasites in certain families of lizards, and the relationships of these parasites in others, seem to be based more upon modifications which have arisen in connection with a certain habit of life than upon considerations of phylogenetic affinity between host families. As has been said elsewhere, the South African families of lizards can, with a few exceptions, be divided into three groups: (*a*) those which have prostigmatic mites, (*b*) those which have mesostigmatic mites, (*c*) those which have neither. The three families of lizards which have no parasites exhibit in each case very different modes of life, being ground-living, burrowing, and arboreal respectively. The

common element in these families is the absence of a definitely imbricating lepidosis, the scales of the Varanidae and Chamaeleontidae being granular or tuberculiform, while those of the vermiform Amphisbaenidae have completely disappeared; in the case of the Varanidae a semi-aquatic habitat may also be a factor. None of these families are related to each other in any way. The four families of lizards which are the hosts of prostigmatic mites form a group which is not phylogenetically homogeneous, the Zonuridae and Gerrhosauridae being quite unrelated to the Geckonidae and Agamidae. They are, however, in South Africa all predominantly rupicolous in habit, with the exception of some of the Gerrhosauridae which are also parasitised by mesostigmatic mites. All these families with a few exceptions (cf. *Platysaurus*) have imbricating scales either dorsally or ventrally which provide mites with an opportunity of fixing themselves beneath them. The Gerrhosauridae with regard to their habitat and the possession of both prostigmatic and mesostigmatic mites, appear to be intermediate between the above-mentioned rupicolous group and the Lacertid-Scincid one, which with a few exceptions consists of scrub, grass, or sand-living forms, but certainly not rock-living ones in the same sense as are the Zonuridae for instance.

With regard to genera, considered as units, another element is introduced. In the Zonuridae, for instance, all the genera with the exception of *Chamaesaura* are rock-living forms "par excellence." In the latter, considered to be the most primitive of the four Zonurid genera, the body has become serpentiform and the limbs reduced so that the animal glides swiftly through grass and shrub; in this genus there are no scale parasites. This may be due not so much to the primitive nature of the genus as to modifications of lepidosis which have kept pace with the lengthening body and limb reductions. These modifications may have taken a direction unfavourable to scale parasites. In the Gerrhosauridae, on the other hand, *Gerrhosaurus*, which lives partly in undergrowth and is partly a burrower, is well provided with parasites, while *Tetradactylus* with a habitat rather like that of *Chamaesaura*, and where the limbs are also reduced, has none. This also occurs in genera of the family Scincidae which have lost their limbs; for instance *Acontias*, *Scelotes*, and *Typhlosaurus*, in which the mesostigmatic mites found on normal forms like *Mabuia* and *Lygosoma* are absent. The process has gone even further in the Amphisbaenidae where both limbs and scales have vanished and where genera parasitised by scale mites are altogether absent.

Taking species alone into consideration it is more difficult to understand why certain forms have escaped parasitism. In the whole of the fairly large family Zonuridae, with the genus *Chamaesaura* excepted, only two species, *Zonurus cataphractus* and *Z. giganteus*, have been found to lack scale parasites. It cannot be urged that insufficient numbers of individual lizards have been examined in the case of these two species. The similarity of lepidosis in these forms may perhaps be an indication that the very coarse strongly spined scales are unsuitable for parasites on account of the greater amount of exposure to

which they would be subjected. Apart from this there seems to be no obvious reason why they should have remained free while the other members of the family are infested.

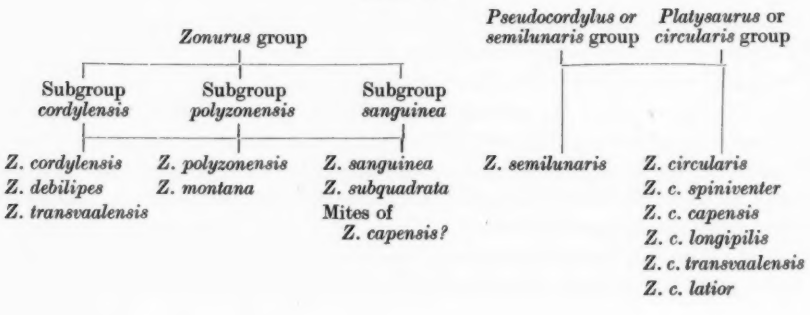
Generally speaking, therefore, mite parasites are absent (i) in families of lizards in which scales are entirely absent or which lack imbricating scales, (ii) in genera where degeneration of the limbs has taken place, whether these degenerate forms are burrowing as in the Scincidae, or surface living as in the Zonuridae and Gerrhosauridae, and (iii) in certain isolated species of genera in which the majority of forms are parasitised.

(*b*) *The correlation of parasite and host in the family Zonuridae*

The large majority of species in this family of lizards, with the exception of the genus *Chamaesaura*, are parasitised by mites of the genus *Zonurobia*. The genus *Zonurus* differs from *Platysaurus* and *Pseudocordylus* in consisting of a large number of species which are markedly distinct from each other in many of their external characters. The two latter genera with a distribution hardly less extensive than that of *Zonurus*, consist of a far smaller number of species, these being characterised by comparatively slight differences of lepidosis, size, etc. The large series of very distinct species in *Zonurus* is undoubtedly due to the sluggish habits of this group which has led to that excessive localisation of species which is so marked a characteristic of the genus. All the species except *Z. cordylus*, *Z. polyzonus*, and to a lesser extent *Z. vittifer* and *Z. jonesi*, occupy a definitely limited area; isolation offers the only adequate explanation of the inclusion in one genus of such widely differing forms as the small smooth scaled *robertsi* at the one extreme, and the large *giganteus*, with its coarse powerfully spined scales, at the other. If the differentiation into species of the parasites runs parallel to the differentiation of the hosts, we should expect to find a larger number of distinct species of parasite on members of the genus *Zonurus* than on *Pseudocordylus* or *Platysaurus*. This is actually the case; for while seven species of *Zonurobia* are found on *Zonurus*, only one is found on each of the genera *Pseudocordylus* and *Platysaurus*; moreover these seven species found on *Zonurus* are morphologically more closely allied to each other than to the other two species. The forms of *Zonurobia* found on *Platysaurus* show far less differentiation, the case being met by giving varietal rank to forms differing from the type. This again affords a parallelism with the host species, which in *Platysaurus* have far fewer distinctive external characters, and which have a tendency to overlap. It must be remembered that in these scale mites where the differentiation of the parasite has, on the whole, kept pace with the differentiation of the host, the mite is largely exposed to fluctuating external conditions, such as temperature and humidity, from which avian parasites, such as Anoplura and Feather-mites, are protected by the constant blood temperature and body coverings of the host.

The parasites of the Zonuridae, taking only the genus *Zonurobia* into consideration, fall naturally into three groups, the *Zonurus*, *Pseudocordylus*, and

Platysaurus groups. These three groups are distinguished by quite different types of structure of the body hairs and the anal region. The characters on which they have been chiefly based are the armature and shape of the anal region, these being considered as of greater value than the shape, size, and arrangement of the body hairs; they have already provided excellent characters for distinguishing closely allied species. The *Zonurus* group consists of several species which have a generalised type of structure in the anal region, quite different from that of the *Pseudocordylus* group with its single species, or from that of the one species and its varietal allies which represent the *Platysaurus* group. Varieties of this single species of the *Platysaurus* group of mites, also parasitise three species of *Zonurus—vittifer, breyeri* and *jonesi*—and these mites have evidently been taken over from *Platysaurus*, as the distributional areas in central South Africa of the three species are more or less coincident with that of *Platysaurus*. *Zonurus peersi* also harbours a few parasites of which *Z. polyzonus* is obviously the true host species. Apart from these exceptional cases, the parasites of the *Zonurus* group can be redivided into three subgroups differing clearly from each other in the structure of the anal field and also of the mouth-parts. The first of these, the *cordylensis* subgroup, contains the mites of mountain living forms extending along the coast from the south-west Cape to the mountain ranges of Zululand and the Eastern Transvaal. In this subgroup the scale mites of the small *Zonurus cordylus* are undoubtedly less closely allied to the two remaining subgroups than to those of the large Zonures which inhabit the Eastern and north-eastern mountain ranges of South Africa, and which again form among themselves a closely interrelated group. The second or *polyzonensis* subgroup consisting of two or three species, is found along the Western coastal belt from the Cape to South-west Africa. The third or *sanguinea* subgroup consists of two isolated centres in the south-western and middle Cape; the parasites of these two peculiar host species, *coeruleopunctatus* and *robertsi*, are very closely allied, not only in structure but also in their habits, and are probably further linked up by the mites of *Zonurus capensis*. These groups and subgroups of parasites can be represented as follows:



If the names of host species are substituted for mite species the following scheme would result:

ZONURIDAE

Zonurus			*Pseudocordylus*	*Platysaurus*
Z. cordylus	*Z. polyzonus*	*Z. coeruleo-punctatus*	*P. microlepidotus*	*P. guttatus*
Z. warreni	*Z. namaquensis*	*Z. robertsi*	*P. fasciatus*	*P. g. minor*
Z. laevigatus		*Z. capensis?*		*P. capensis*
Z. vandami				*P. wilhelmi*
Z. v. perkoensis				*Z. vittifer*
Z. barbertonensis				*Z. breyeri*
depressus				*Z. jonesi*

Whether this arrangement of the hosts has any validity as an expression of the relations between the various species of *Zonurus* it is at the moment not possible to say, but in view of the lack of unanimity between systematic writers as to the real relationships of the species, it may prove suggestive when an attempt is made to distinguish characters of paleotelic value from secondary specialisations.

(c) *List of prostigmatic mites parasitic on South African Zonuridae and Gerrhosauridae*

The following list contains the known species of South African Zonuridae and Gerrhosauridae and their prostigmatic mite parasites. In the family Zonuridae no specimens of *Zonurus pustulatus*, *Z. cordylus rhodesianus* and *Z. cordylus tasmani* have been examined. Five specimens of *Z. macropholis* and four of *Z. capensis* have been investigated with negative results. All these forms may be found to possess parasites when adequate numbers have been examined.

Family ZONURIDAE

Genus	Species	Parasite
Zonurus	*Z. warreni*	*Zonurobia debilipes*
	Z. vandami	*Zonurobia transvaalensis*
	Z. vandami perkoensis	*Zonurobia transvaalensis*
	Z. laevigatus	*Zonurobia transvaalensis*
	Z. barbertonensis	*Zonurobia transvaalensis?*
	Z. barbertonensis depressus	*Zonurobia transvaalensis*
	Z. cordylus	*Zonurobia cordylensis*
		Scaphothrix convexa
	Z. cordylus rhodesianus	—
	Z. cordylus tasmani	—
	Z. polyzonensis	*Zonurobia polyzonensis*
		Scaphothrix convexa
		Ixodiderma inverta
	Z. peersi	*Zonurobia polyzonensis*
		Scaphothrix convexa
	Z. namaquensis	*Zonurobia montana*

Genus	Species	Parasite
Zonurus	*Z. coeruleopunctatus*	*Zonurobia sanguinea*
		Ixodiderma inverta
	Z. robertsi	*Zonurobia subquadrata*
	Z. vittifer	*Zonurobia circularis latior*
	Z. breyeri	*Zonurobia circularis latior*
	Z. jonesi	*Zonurobia circularis*
	Z. capensis	—
	Z. macropholis	—
	Z. pustulatus	—
	Z. giganteus	None
	Z. cataphractus	None
Pseudocordylus	*P. microlepidotus*	*Zonurobia semilunaris*
		Ixodiderma inverta
	P. microlepidotus namaquensis	—
	P. fasciatus	*Zonurobia semilunaris*
		Ixodiderma inverta
	P. subviridis	*Ixodiderma inverta*
		Ixodiderma pilosa
Platysaurus	*P. guttatus*	*Zonurobia circularis typicus*
	P. guttatus minor	*Zonurobia circularis spiniventer*
	P. capensis	*Zonurobia circularis capensis*
	P. wilhelmi	*Zonurobia circularis transvaalensis*
Chamaesaura	*C. anguina*	None
	C. aenea	None
	C. macropholis	None

Family Gerrhosauridae

Gerrhosaurus	*G. flavigularis*	*Pterygosoma bicolor*
		Pterygosoma hystrix
	G. f. nigrolineatus	*Pterygosoma bicolor*
	G. auritus	*Pterygosoma bicolor*
	G. validus	*Pterygosoma gerrhosauri*
	G. typicus	—
	G. major grandis	*Pterygosoma gerrhosauri*
Cordylosaurus	*C. trivittatus australis*	None
	C. trivittatus	None
	C. subtesselatus	None
Tetradactylus	*T. tetradactylus*	None
	T. seps	None
	T. breyeri	None
	T. africanus	None
	T. bilineatus	None
	T. laevicauda	None
	T. fitzsimonsi	None
	T. eastwoodae	None

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(*MS. received for publication* 12. III. 1934.—Ed.)

ON *TRYPANOSOMA BRUCEI*, *T. RHODESIENSE* AND *T. GAMBIENSE* AND THEIR ABILITY TO INFECT MAN

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Introduction

Ever since the discovery of cyclical development of the trypanosomes of man in *Glossina*, it has been generally assumed that this so-called biological method of transmission accounts for the distribution and spread of human trypanosomiasis in Africa.

Fourteen years ago W. F. Fiske and I called attention to the possible importance of direct transmission in epidemic trypanosomiasis, basing our views on the evidence afforded by the records of the great Uganda epidemic that raged during the early years of this century (Duke, 1919). Later, in 1923, after a visit to the Mwanza infected area to the south of Lake Victoria where *T. rhodesiense* was spreading by the agency of *G. swynnertoni*, I drew attention to certain features of that epidemic that seemed to favour the operation of direct transmission from man to man by tsetse (Duke, 1923*a*). In papers on the zoological status of the polymorphic trypanosomes the two methods of transmission were contrasted and discussed (Duke, 1921, 1923), especially in relation to the phylogeny of *T. rhodesiense*. Taylor in Nigeria (1930) has also directed attention to the possibility of direct transmission of man's trypanosomes.

On the other hand, in the last few years important additions have also been made to our knowledge of the effect on these parasites of cyclical passage through *Glossina*.

It has long been believed that cyclical transmission through tsetse stabilises a trypanosome by eliminating acquired abnormalities. Supposing that *T. brucei* possesses a latent power of infecting man, and that sometimes in nature it exercises this power, the result would be, according to our present system of nomenclature, *T. rhodesiense*. Would the ability to use man as a host survive the passage of the strain through the *Glossina*?

Evidence on the effect on a trypanosome of cyclical passage through *Glossina* has been gradually accumulating in recent years. Schilling and Schreck (1930), and quite recently Lester (1933) have recorded a diminution in virulence following biological development in the fly. The German authors went much further and reported that even the serological characters of a strain

were changed by a single cyclical passage. Yorke and his collaborators (Yorke *et al.* 1930) and Van Hoof and Henrard (1933) have shown that the resistance of a trypanosome to arsenicals, whether experimentally intensified or inherited, survives unchanged the biological passage through the fly; and Lester has found the same for acquired resistance against human serum.

Adams (1933), working at this Institute and employing the Liverpool technique, examined the resistance to human serum of four strains of *T. rhodesiense* recently isolated from man. He found that in the first passage from man these strains possessed a definite resistance to the serum, but that all four became serum-sensitive when they were maintained by passage through guinea-pigs. With these four strains the loss of resistance became manifest at the sixth, fifth, eighth and third passage respectively. Six other strains all proved sensitive to human serum when first examined. Adams also found that after a single cyclical passage through *Glossina* the resistance *in vitro* of a strain to human serum was reduced, whereas in controls where the strain was passaged by the direct method this reduction was not observed. He found, too, that the sensitiveness to human serum of two strains of *T. brucei* was no greater than that exhibited by *T. rhodesiense* strains that had been kept up for a few months in laboratory animals. In striking contrast to the behaviour of *T. rhodesiense*, *T. gambiense* was found both at Liverpool and by Adams in Uganda to retain its resistance to human serum for long periods of maintenance at the laboratory.

In a recent paper I have shown that repeated cyclical passage of a strain of *T. rhodesiense* or *T. gambiense* through the *G. palpalis* of Lake Victoria certainly does not enhance and may even be attended by a diminution of transmissibility (Duke, 1934).

A perusal of the meagre experimental evidence accumulated on this subject reveals the interesting fact that there is no record of the experimental infection of man by cyclically infected *Glossina*, either with *T. gambiense* or *T. rhodesiense*. It is true that the occurrence of inflammation at the site of a tsetse bite has been noted, this symptom apparently being associated with the onset of infection in man. But this is presumption, not proof, of infection by a cyclically infected fly. Corson (1932) came very near to experimental demonstration when he fed upon himself *G. morsitans* infected with *T. rhodesiense*, but at the last moment he introduced a syringe injection of infected blood and so obscured the issue.

In this paper will be found what is, then, the first record of the experimental infection of man either with *T. gambiense* or with *T. rhodesiense* by the bite of a cyclically infected tsetse. It also contains an account of the loss by two accredited strains of *T. rhodesiense* of the power to infect man. These latter experiments were carried out in connection with a still unfinished investigation of the antelope reservoir of man's trypanosomes, about which a report is in course of preparation. They are published here because they do not essentially concern the reservoir problem, and can, therefore, appropriately

be considered independently. Both these strains of *T. rhodesiense* have also been studied in antelope for a considerable time and their attitude to man fully examined. The results appear later in this *Journal* (Duke, 1935).

The fact that *T. rhodesiense*, under conditions that may reasonably be described as natural, may lose its power of infecting man is of considerable interest. This possibility was to some extent foreshadowed by the work of Yorke, Adams, and Murgatroyd on the effect of human serum on man's trypanosomes *in vitro* (Yorke *et al.* 1930). But the only reliable criterion in this matter is, unfortunately, the test on the living human subject, applied as nearly as possible in the way nature applies it. As remarked elsewhere, the discovery of this phenomenon of loss of pathogenicity to man does not itself justify the separation zoologically of *T. rhodesiense* from *T. gambiense*—although their ability to infect man is their principal if not sole point of resemblance—until, at all events, we know whether *T. gambiense* behaves in the same manner in similar circumstances. It will be seen below that the only experiment hitherto performed at Entebbe with *T. gambiense*, that is in any way comparable with the treatment applied here to *T. rhodesiense* Strain Tinde III, gave a strikingly different result.

Much, indeed, remains to be done, but it is hoped that the results here described, together with the additional evidence afforded by the antelope reservoir investigation, will help to a better understanding of these trypanosomes and their relations both to one another and to man.

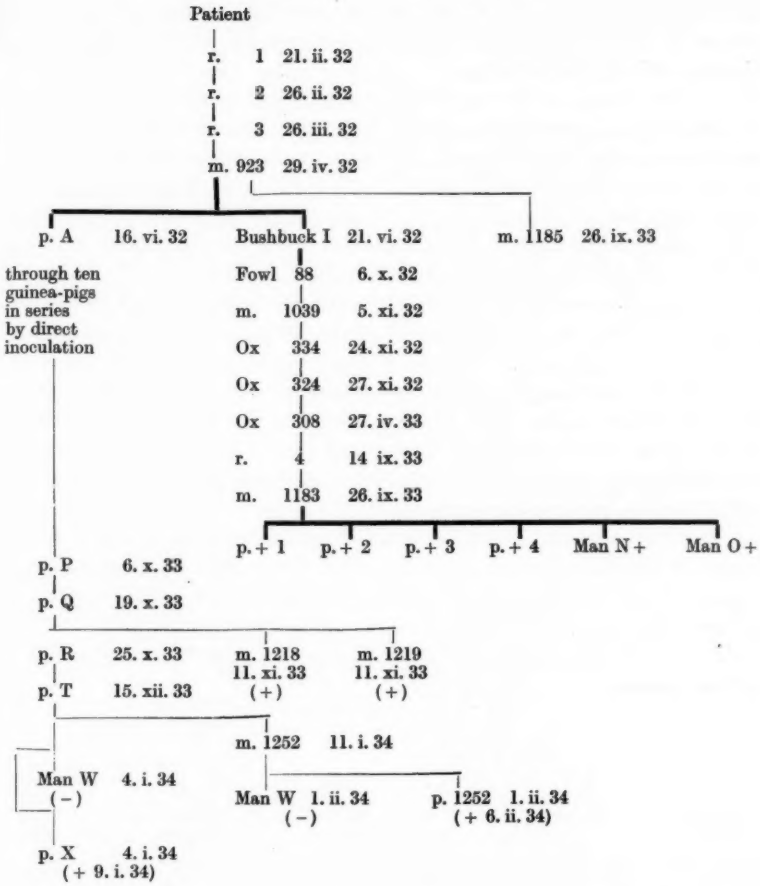
Experimental

In reporting the results of experiments of this kind there is a great deal of detail, much of it instructive, that must of necessity be excluded. Thus the detailed history of the individual boxes of flies that go to make a test is omitted, each test being presented in the form of summary. In these summaries certain data are given, always in the same order. Thus reading from left to right the figures represent the period covered by the test; the total number of flies dissected; the number of these found to contain flagellates; the percentage of infected flies; the number of gland-infected flies; the number of infected flies living 25 days or longer; and lastly, the *transmissibility index* (Duke, 1928). The full headings will be given in Table I, after which in subsequent summaries they will be omitted.

The various strains examined will now be considered.

(1) *Strain Tinde III. T. rhodesiense*, from man, sent to the Institute by Dr H. Fairbairn from northern Tanganyika Territory.

The maintenance of this strain is shown in the accompanying schedule. In this and the other schedules p. = guinea-pig, m. = monkey, r. = white rat. The + sign means infection with trypanosomes, the − sign failure to become infected. A thin line between two consecutive animals shows a syringe passage; a thick line, a passage by cyclically infected *Glossina*. In all these experiments *G. palpalis* is the vector.


Patient
r. 1 21. ii. 32
r. 2 26. ii. 32
r. 3 26. iii. 32
m. 923 29. iv. 32
p. A 16. vi. 32
Bushbuck I 21. vi. 32
m. 1185 26. ix. 33
through ten guinea-pigs in series by direct inoculation
Fowl 88 6. x. 32
m. 1039 5. xi. 32
Ox 334 24. xi. 32
Ox 324 27. xi. 32
Ox 308 27. iv. 33
r. 4 14 ix. 33
m. 1183 26. ix. 33
p. + 1
p. + 2
p. + 3
p. + 4
Man N +
Man O +
p. P 6. x. 33
p. Q 19. x. 33
p. R 25. x. 33
m. 1218 11. xi. 33 (+)
m. 1219 11. xi. 33 (+)
p. T 15. xii. 33
m. 1252 11. i. 34
Man W 4. i. 34 (−)
Man W 1. ii. 34 (−)
p. 1252 1. ii. 34 (+ 6. ii. 34)
p. X 4. i. 34 (+ 9. i. 34)

It will be seen that two series of passages were carried out, one through guinea-pigs and the other through a bushbuck, a fowl and three oxen. The guinea-pig series commenced with the infection of pig A by fly on 16. vi. 32, and thereafter the strain was passed by the syringe through a succession of fourteen guinea-pigs over a period of 18 months. It was then tested on man by inoculating subcutaneously into volunteer W $\frac{3}{4}$ c.c. of a strong suspension of trypanosomes in sodium citrate solution. It will be noted that two different tests on man were performed: in the first the infected blood came from guinea-pig T; in the second, from a monkey, 21 days after its first infection. In each case the control guinea-pig became infected after an incubation period of only 5 days.

In the ox series the trypanosome, after 98 days in a bushbuck, was passed cyclically by fly to a fowl and thence by the syringe, *via* a monkey, into the

first ox of the series. The strain remained in oxen for 294 days. It was then inoculated by the syringe *via* a white rat into monkey 1183, from which were infected the flies that infected man.

At the end of each of the series of passages a test of the transmissibility of the strain by *G. palpalis* was carried out. The difference between the two results is remarkable and is shown in Table I, which gives a number of transmissibility tests applied to this strain in various animals. It will be seen that after passage through the guinea-pigs the infectivity of the strain to *G. palpalis* was diminished enormously, whereas residence in the oxen produced no such effect. This is interesting in the light of previous experience of the effect on man's trypanosomes of sojourn in calves, the indices of a strain examined in the blood of the calf being invariably reduced (Duke, 1934). In the present instance it is evident that if any such inhibitory effect was induced by the tissues of the oxen it was only temporary, the transmissibility of the strain in monkey 1183 being actually higher than in the majority of the other tests. Another point of interest to be noted in passing is the fact that a stay of 98 days in the bushbuck (and, incidentally, 30 days in a fowl) did not apparently affect the ability of this strain to infect man.

Pending the publication of fuller details in the later report on the antelope reservoir, in which this strain figures prominently, it may be remarked here that strain Tinde III in antelope retained its power of infecting man for a longer time than any of the other strains examined in these animals.

Table I shows the transmissibility tests carried out with strain Tinde III in various animals.

Table I

Infecting animal	Period covered by test	Total flies dissected	Total infected flies	Infected flies %	Number of infected flies alive at 25th day: With glands infected	Number of infected flies alive at 25th day: Total	Transmissibility index
Monkey 923	15. v.–5. vi. 32	440	30	6·8	15	17	*6*
Bushbuck I	12–17. ix. 32	488	18	3·6	2	15	*0·4*
Monkey 1140	7. vi.–20. vii. 32	378	12	3·1	1	2	*1·5*
,, 1154	17–20. vii. 32	152	4	2·6	1	2	*1·3*
,, 1157	25–31. vii. 33	366	13	3·5	2	6	*1·1*
,, 1162	5–14. ix. 33	520	8	1·5	5	5	*1·5*
,, 1185	4–16. x. 33	454	18	3·5	9	10	*3·1*
,, 1183	3–13. x. 33	390	21	5·3	15	20	*3·9*
,, 1213	27. xii. 33–5. i. 34	169	6	3·5	1	2	*1·7*
,, 1218–9	17–27. xi. 33	533	1	0·1	0	1	*0*

(2) *Strain Tinde I. T. rhodesiense*, of similar origin to strain Tinde III. The history of this strain is shown in the schedule on p. 51.

Transmissibility tests performed with strain Tinde I on man

(*a*) Monkey 1153, representing the tenth cyclical passage from monkey 949, these passages occupying a period of some 15 months. The summary of the transmissibility test on monkey 1153 is as follows:

22–28. vii. 33	487	22	4·5	11	15	*3·3*

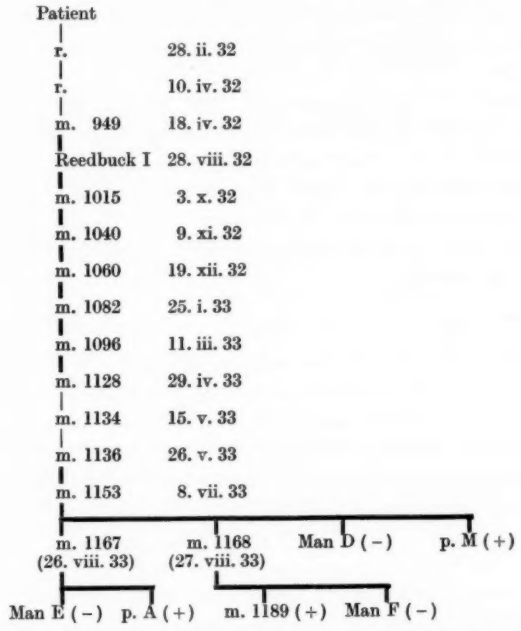
These flies infected a monkey before and several guinea-pigs both before and after they fed upon the volunteers D, E and F.

The last infective bite on volunteer D was on 2. ix. 33; on volunteer E, 28. viii. 33; and on volunteer F, 31. viii. 33.

(*b*) Monkey 1167, representing the eleventh cyclical passage from monkey 949. Only a few flies were employed on this monkey, the summary of the test being:

3–10. ix. 33	90	4	4·4	2	2	*4·4*

These two gland-infected flies infected guinea-pigs before they fed on man: they both fed upon volunteer E, for the last time on 9. x. 33.



(*c*) Monkey 1168, also representing the eleventh cyclical passage from monkey 949. The summary of the transmissibility test on this monkey was:

3–22. ix. 33	422	10	2·3	2	5	*0·9*

These two gland-infected flies infected a monkey before they bit volunteer F: they last fed on the volunteer on 12. x. 33.

On 3. x. 33, 10 c.c. of blood from each of these three volunteers was inoculated subcutaneously into a separate clean monkey: none of these monkeys became infected. The three natives remained in perfect health, and after an adequate interval, two were used for other experiments, in the course of which they became infected. The third volunteer, D, served subsequently

in several negative tests and eventually, late in March 1934, was infected by *T. rhodesiense*, strain LX.

Note. The possibility of delayed or cryptic infections occurring in these volunteers has not been overlooked, and this subject has been discussed in a recent paper (Duke, 1934*b*). From the general behaviour of the men who have become infected it is improbable that infections of this kind do occur with *T. rhodesiense*. Nevertheless in several cases blood inoculations have been made into monkeys from volunteers who have escaped infection, and none of these animals became infected. The results obtained by James, Nicol and Shute (1927), James (1931) and by Swellengrebel and his colleagues (1929) show that infections of this kind do occur in malaria when the cyclical method of infection is used. With the volunteers used at Entebbe all those who became infected complained of malaise about 9–12 days after the infecting bite, and developed high fever (105° F.) as an initial symptom.

(3) *Strain LIII. T. brucei*, recovered from wild *G. morsitans* near the Kazinga channel, between Lakes Edward and George in the Western Province of Uganda; supplied by the Veterinary Pathologist, Uganda.

The transmissibility of this strain was examined in the monkey representing the seventh passage of the trypanosome from the dog in which it was originally isolated (six of these passages were effected cyclically by *G. palpalis*). The summary is as follows:

184	5	2·7	1	2	*1·3*

The single gland-infected fly, having first infected a clean monkey, fed upon volunteer D (20. x. 32) without producing infection.

(4) *Strain LV. T. brucei*, recovered from wild *G. morsitans* along the Victoria Nile, near the Murchison Falls in the Northern Province of Uganda.

The following is the summary of the transmissibility test on this strain in the monkey originally infected by the wild fly:

137	11	8·0	4	7	*4·5*

All four of these gland-infected flies fed on volunteer A (1) without infecting him; they had previously infected a clean monkey.

(5) *Strain LVI (a, b and c). T. brucei*, recovered from the wild *G. palpalis* of Damba Island, Lake Victoria.

The examination of this strain has already been fully reported (Duke, 1932). The conclusions are quoted here to complete the series of tests on man performed with cyclically infected tsetse: "Laboratory-bred *G. palpalis* cyclically infected with three different strains of polymorphic trypanosomes recovered from the wild *G. palpalis* on Damba Island, Victoria Nyanza, have been fed upon four normal human beings, three natives and one European. One native was in this way exposed to infection by all three strains, the other three volunteers to two strains each. None of these volunteers became infected."

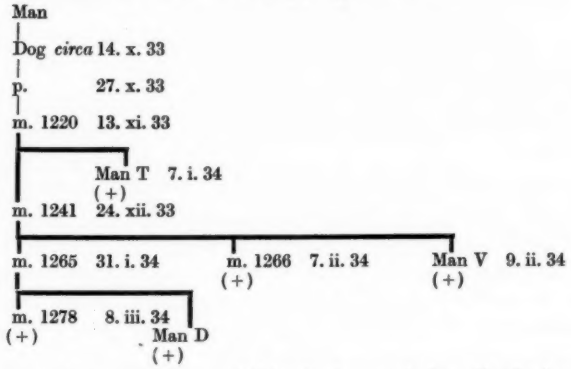
(6) *Strain LVII. T. brucei*, recovered from wild *G. pallidipes* along the Busoga shores of Lake Victoria.

The following is the summary of the transmissibility test on this strain in the monkey infected by the wild fly:

167	11	6·5	4	6	*4·3*

Certainly one and probably three of these gland-infected flies fed on a volunteer B (on 10. x. 32) without infecting him: these flies had previously infected a clean monkey.

(7) *Strain LX. T. rhodesiense*, recovered from a native of the Buzinja country south of Lake Victoria, Tanganyika Territory. This man was found in an advanced state of the disease near masaka in south-west Uganda. The strain was first isolated in a dog, which was sent to the Institute. The subsequent history is shown in the accompanying schedule:



Three successive generations of this strain, carried cyclically by *G. palpalis* from animal to animal, proved readily infective to man. The incubation periods in the volunteers were as follows:

Volunteer	T	...	...	13 days
,,	V	...	...	11 ,,
,,	D	...	...	11 ,,

In each case trypanosomes were found on the first day the volunteer complained of feeling indisposed: they would probably have been discernible at least a day earlier.

Transmissibility tests

m. 1220	27. xi.–8. xii. 33	373	11	2·9	6	6	*2·9*
m. 1241	4–15. i. 34	463	8	1·7	6	6	*1·7*
m. 1265	8–21. ii. 34	383	16	4·1	9	15	*2·4*
m. 1266	15. ii.–1. iii. 34	556	24	4·3	13	16	*3·4*

[*Note.* The infection of man with this strain and with strain LX was carried out in connection with an investigation, still in progress, on the prophylactic effect of Bayer 205 (Duke, 1934*c*).]

(8) *Strain LXI. T. gambiense*, isolated from a Uganda native on 27. vii. 33

by subinoculation of the man's blood into a monkey. The summary of the transmissibility test carried out on this strain is as follows:

539 21 3·8 14 16 3·3

Two of these gland-infected flies fed upon volunteer P on 7. xi. 33 and infected him, trypanosomes appearing in the man's blood 9 days later.

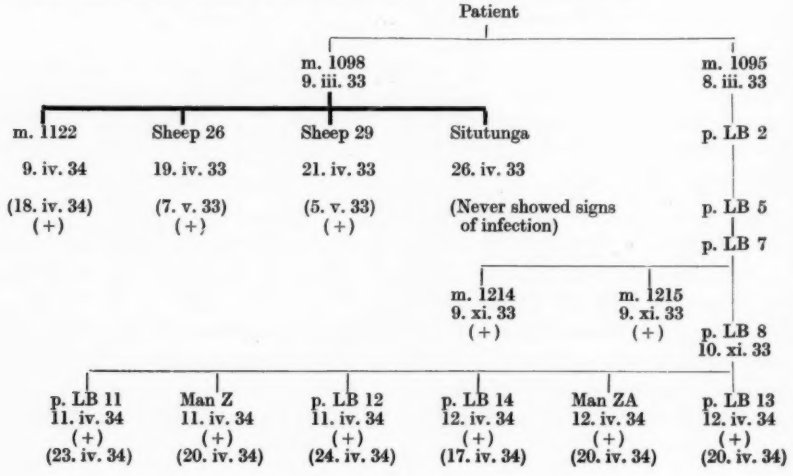
(9) *Strain "Braun." T. gambiense*, brought to Uganda from Prof. Reichenow's laboratory, at the Tropical Diseases Institute at Hamburg, late in 1931. Isolated from a patient from Fernando Po on 25. ii. 20 at Hamburg, and maintained ever since in laboratory rodents. Since reaching Entebbe it has been passaged by the syringe through a guinea-pig, a monkey, and finally a series of fourteen guinea-pigs. Citrated blood from the last of these guinea-pigs was inoculated subcutaneously on 15. ii. 34 into volunteer H and into a clean guinea-pig. Both the volunteer and the guinea-pig showed trypanosomes on the sixth day after the inoculation. It is worth noting that some 4 months previously volunteer H had been bitten but not infected by five flies whose glands were infected with *T. rhodesiense*, strain III. This latter strain had suffered impairment but not complete loss of its power to use man, so this observation shows that volunteer H was not, at all events, abnormally sensitive to trypanosomes.

The transmissibility of Strain Braun was examined at intervals between November 1931 and April 1933. 1137 laboratory-bred *G. palpalis* were employed of which only two became infected. These two flies died on the 18th and 31st day respectively after their feed on the infected monkey, and in neither did the salivary glands contain any flagellates (Duke, 1934 *a*).

(10) *Strain LII. T. gambiense*, from Katwe area, Lake Edward, Western Uganda.

The strain was isolated in monkeys 1095 and 1098 which received subcutaneously citrated blood of the patient on 8 and 9. iii. 33 respectively.

The maintenance of the strain since its isolation is as follows:



Transmissibility tests with Strain LII

m. 1095, 1098	19–30. iii. 33	590	26	4·4	19	21	*3·3*
m. 1214,1215	28. xi.–12. xii. 33	603	13	2·1	0	9	*0*
Sheep 26	30. v.–14. vi. 33	362	5	1·3	3	5	*0·7*
Sheep 29	30. v.–20. vi. 33	426	2	0·5	1	1	*0·4*
Sheep 26	9–25. i. 34	577	4	0·7	1	2	*0·3*

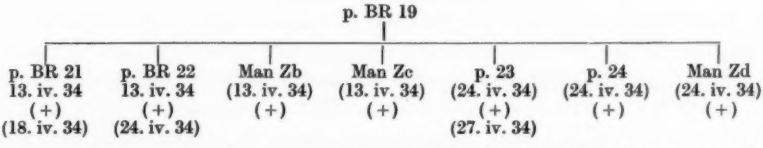
A year after the first isolation of the strain from man, volunteers Z and ZA were inoculated subcutaneously with ¾ c.c. citrated blood of guinea-pig LB 8, which was showing on each occasion about six trypanosomes per field under 1/6 in. objective and 2 eyepiece. The incubation periods in the two men were 9 and 8 days.

The situtunga never showed any sign of infection in stained thick blood films, and 10 c.c. of its blood mixed with citrate failed to infect a clean monkey on 20. vii. 33. In sheep 26 this strain remained transmissible for at least 9 months.

(11) *Strain BR. T. gambiense*, isolated from a European on 14. vi. 30 and brought out to Uganda at the end of 1931. The transmissibility of this strain has been dealt with elsewhere (Duke, 1934 *a*). Prof. J. G. Thomson, who kindly let me have this strain, states that it appears to him to be half-way between typical *T. gambiense* and typical *T. rhodesiense*.

At Entebbe the strain was kept up in guinea-pigs by direct passage. The tests on man were as follows: in each case the volunteer received subcutaneously ¾ c.c. citrated blood of a guinea-pig in which trypanosomes were very numerous.

Schedule of maintenance



Through a mistake, the two men Zb and Zc both received an intravenous injection of 1 g. tryparsamide on 21. iv. 34 before trypanosomes had actually been demonstrated in their blood, although local and general symptoms were severe at the time. Trypanosomes were first found in both men by gland puncture on 27. iv. 34, and they were promptly treated with Bayer 205. This indifference to tryparsamide agrees with Prof. Thomson's description of this strain as possessing some of the attributes of *T. rhodesiense*.

This strain was still pathogenic to man three years and ten months after its first isolation from his blood.

Discussion

We will now discuss the above results and see what instruction they convey.

To take first of all the effect of the guinea-pig passages on strain Tinde III. Before the guinea-pig series began, the strain was both transmissible by tsetse and, judging from the behaviour of the other series in the schedule, also pathogenic to man. After fourteen passages over a period of 18 months both these attributes had been lost. Long experience of the transmission of trypanosomes has gradually convinced me that maintenance of a strain in guinea-pigs, even for a short time, is liable to impair its cyclical transmissibility by tsetse. My colleague, Dr J. M. Wallace, has had the same experience; and quite recently Van Hoof and Henrard, though on evidence perhaps somewhat scanty for a final pronouncement, have reached the same conclusion (Van Hoof and Henrard, 1933).

The loss of pathogenicity to man by strain Tinde III is the first substantiated record of this phenomenon. The change is apparently ascribable to the effect of passage through guinea-pigs, for the same strain under different conditions retained this property[1]. It underwent no apparent change in morphology. Corson (1932) found that a strain of *T. rhodesiense* which had been maintained in goats for 19 months by direct inoculation was at the end of that time still pathogenic to man. In a later study on the resistance of trypanosomes to human serum he speaks of "the increased susceptibility [*i.e.* to human serum] caused by guinea-pigs," but continues "unless, as is possible, this [*i.e.* increased susceptibility] was acquired in the flies" [*i.e.* in the course of cyclical development] (Corson, 1933). And in the earlier paper he remarks "It appears as if passage through tsetse fly into a guinea-pig had resulted in a somewhat lessened susceptibility [*i.e.* to human serum] but the experimental evidence is too small to base conclusions on."

There is thus some published evidence that the maintenance of a trypanosome in guinea-pigs has an unfavourable effect on certain of its activities, reducing its transmissibility by *Glossina* and—though this is less certain—impairing its ability to infect man.

The schedules in this paper also supply some definite information about the power of a trypanosome to infect man by the cyclical method. He has now been experimentally infected with both *T. gambiense* (strain LXI) and *T. rhodesiense* (strain LXII) by cyclically infected *G. palpalis*, and the latter trypanosome has retained its pathogenicity to man apparently unimpaired by three successive cyclical passages through *G. palpalis*.

Another interesting point is the loss of their power to infect man by two strains of *T. rhodesiense* while actually under observation. In the schedule of strain Tinde III it will be seen that at the end of the series of guinea-pig passages both pathogenicity to man and transmissibility by *Glossina* were lost, whereas the sojourn in oxen, preceded and followed by cyclical passage through the fly, had no apparent effect on either quality. At first sight this remarkable

[1] Altogether, in various experiments with this strain, nine volunteers have been infected.

contrast disposes of the criticism that the strains of *T. rhodesiense* received from Tinde owed their establishment in man in the first place to the patients from whom they were isolated being abnormally sensitive to trypanosomes, so that their subsequent failure to infect normal man would not be surprising. If this criticism is to apply to strain Tinde III it is necessary to assume that the volunteer W was possessed originally of an abnormally high resistance to trypanosomes, for other branches of this strain infected altogether nine volunteers. This possibility, that people differ in their power of resisting trypanosomes, is indeed one of the difficulties to be faced in this research, and to cover the point satisfactorily by experiment it would be necessary to employ a number of volunteers far beyond the present supply. Yet another point to be borne in mind is the possible protecting effect in man of repeated bites by gland-infected flies carrying trypanosomes not pathogenic to man, and on this subject there will be something to say in a later report, in which, also, the subsequent behaviour of volunteer W will be described (Duke, 1935). It is sufficient to mention here that this man and two others whose behaviour suggested the possession of unusual resistance were subsequently proved to be susceptible to infection with *T. rhodesiense*. Before leaving this strain it is worthy of note that Adams, using the *in vitro* method, found evidence that cyclical passage through *G. palpalis* coincided with a reduction in the resistance of a strain to human serum (Adams, 1933).

We now come to the striking contrast between the behaviour of the Hamburg strain of *T. gambiense* (No. 9) and that of strain Tinde III in guinea-pigs. Prolonged residence in small laboratory animals, *extending over some 14 years and involving an enormous number of passages*, has failed to impair the pathogenicity of the former strain for man, and it is noteworthy that the volunteer (H) used for this test had some 4 months previously survived heavy exposure to the metacyclic forms of *T. rhodesiense* (strain Tinde II), so that he cannot be described as hypersensitive. On the other hand, this Hamburg strain has completely lost its transmissibility by and almost completely its infectivity to *G. palpalis* (Duke, 1934*a*).

These observations are very suggestive. They indicate first of all the relative antiquity of *T. gambiense* as a parasite of man. They point also with equal clearness to the comparative instability of the power of *T. rhodesiense* to infect man, and show how easily this trypanosome may revert to a form indistinguishable from typical "wild" *T. brucei*, the seemingly harmless parasite of the big game of Africa. Once again it must be noted that the quality of pathogenicity to man is, according to all the available evidence, deeply rooted in the constitution of *T. gambiense*.

Kleine and his co-workers believe that *T. gambiense* and *T. rhodesiense* are one and the same species, zoologically distinct from *T. brucei* and characterised by its power to infect man. But we have here a strong indication not only that there is a great difference between the two trypanosomes of man in this important respect, but also definite proof that pathogenicity to man is not entitled to specific significance.

Kleine believes that *T. rhodesiense* is merely *T. gambiense* in an unfamiliar environment, and that, given time, it will settle down again in man and revert to the *gambiense* type. I agree with Kleine that if *T. rhodesiense* is left in contact with man in nature it will gradually conform to the *gambiense* pattern. This is how, in my view, *T. gambiense* itself has evolved in past ages from *T. brucei*: in its typical form *T. gambiense* represents the highest degree of adjustment yet attained between *T. brucei* and man. According to Kleine's view there is no *T. rhodesiense* without the intervention at some time or other of *T. gambiense* in man himself, this latter trypanosome being supposed to retain tenaciously the power of using man. Kleine ascribes the greater virulence of *T. rhodesiense* to contact with a non-immune population. If this were so "*T. rhodesiense*" would surely appear wherever *T. gambiense* attained epidemic proportions. It has still to be determined whether *T. gambiense* can in the course of a few years assume the characters of *T. rhodesiense*, as a result of changing its environment from *G. palpalis* (or *G. tachinoides*) and man to *G. morsitans* and game. If all three trypanosomes are really one species, this change is to be expected, although it may well require a considerable time to come about. The trypanosomes of Damba Island, Lake Victoria, which I believe to be the descendants of the *T. gambiense* of the great Uganda epidemic, after 13 years in antelope and away from all contact with man were still more like to *T. gambiense* than to *T. brucei* from game tsetse areas; but on Damba *G. palpalis*, and not a game tsetse, has always been the sole vector. If once *T. gambiense* changes into *T. rhodesiense* we have seen how easy is the further step to the *brucei* phase, where pathogenicity to man is an abnormal instead of a normal feature of the trypanosome. There is no doubt that Kleine's theory of origin affords a satisfactory explanation of the presence of *T. rhodesiense* in an area such as Nigeria where there is a heavy incidence of *trypanosomiasis gambiensis*, and two and sometimes three species of tsetse. The early Uganda records show that some of the strains of *T. gambiense* at that time caused a rapidly fatal disease in man (Duke, 1919), and it is possible and indeed probable that a close examination would have revealed a resemblance between such strains and the *T. rhodesiense* of to-day. But in areas such as the Luangwa Valley, where the early studies of *T. rhodesiense* were made, it seems to me more difficult to reconcile the view of descent from *T. gambiense* with the state of affairs obtaining at the present day. In the area surrounding the Serenje-Fort Jamieson road and in other parts of the valley the risk of infection to human visitors, black or white, is, I understand, almost as great as the risk a dog ran in 1914 in crossing the now extinct *morsitans* belt between Masindi and the Nile in Uganda. Natives travel far nowadays, and *T. gambiense* is readily transmissible by *G. morsitans*, and there must at times be mixed infections of *T. brucei* and *T. gambiense* in game and possibly in wild fly. But the gap, zoologically speaking, between *T. gambiense* and *T. rhodesiense* is much greater than that between *T. rhodesiense* and *T. brucei*, and we have now seen that the distinction between the two latter is purely arbitrary, and rests on the exaggerated

importance hitherto attached to pathogenicity to man. Now that this character is known to be neither absolute nor stable, there is no longer justification for the common assumption that every strain of *T. rhodesiense* found in man has necessarily had previous knowledge of man.

It is a comparatively simple matter to determine the effect on *T. gambiense* of residence in game animals and their tsetses, and it is to be hoped that this aspect of the reservoir investigation will some day receive the attention it demands and deserves. An essential requisite is an adequate supply of clean game tsetses, which is unfortunately not at the disposal of the Institute. Up to date the available resources at Entebbe have been applied almost exclusively to the study of *T. rhodesiense*, because this species was the obvious starting-point in an investigation of the game reservoir. In the two antelope hitherto exposed to infection with *T. gambiense*, trypanosomes either did not appear at all or the infection died out rapidly. The research carried out by the Royal Society's Commission at Mpumu showed that *T. gambiense* may retain its transmissibility by *G. palpalis* for at least 10 months in antelope, but at that time no attempt was made to determine what effect this experience would have on the power of the trypanosome to infect man (Duke, 1912).

The important point is whether *trypanosomiasis rhodesiensis* ever appears in a game tsetse region without the previous introduction into that area of *T. gambiense*. If strains Tinde I and III are descended from this source, then it follows from the evidence that these descendants, fresh from contact with man, can in from 14 to 17 months lose their infectivity to man—and the change may in each case actually have been completed in a much shorter period. As already remarked, it would be instructive to carry out experiments along these lines. The evidence supplied by tests *in vitro* on *T. gambiense* suggests that this trypanosome clings tenaciously to its power to infect man.

Kinghorn and Yorke in their early investigations in Rhodesia found at least 16 per cent. of the game infected with trypanosomes indistinguishable by ordinary laboratory tests from either *T. brucei* or *T. rhodesiense* (*Reports of the Luangwa Valley Commission*). Believing in the identity of these two organisms they inferred that 16 per cent. of the game carried the human trypanosome. The Germans found the same incidence of infection in another game area hard by, but held that the trypanosome was *T. brucei*, and therefore, according to their views, harmless to man. Kleine criticised Kinghorn and Yorke's conclusions (Kleine, 1923). Why, he asked, is there no mention of *T. brucei* in their calculations? This trypanosome must have been present, and it has at least an equal claim with *T. rhodesiense* to consideration.

In a lively and most interesting discussion on the rôle of the game in Rhodesia in perpetuating trypanosomes pathogenic to man, a debate held by the Royal Society of Tropical Medicine and Hygiene in 1920, Prof. Yorke made his views clear (Yorke, 1920). He maintained, with Bruce and his coworkers in Nyasaland, that *T. rhodesiense* was merely a variety of *T. brucei* that had secured a footing in man; that man was normally completely re-

sistant to *T. brucei*, but that individuals existed whose normal defences against this trypanosome were defective, and in these persons infections might occur. Clearly at that time he did not believe, as might have been concluded from the Luangwa report, that all *T. brucei* strains carried by the game were capable of infecting normal man. He held that the game was the great reservoir from which man becomes infected. To illustrate his point he cited Taute's experiments carried out in 1913, remarking that these experiments merely proved that man is very resistant to infection with the polymorphic trypanosomes of game: "If in his first experiment Taute had fed his laboratory-bred flies on an infected man instead of monkeys and antelope, he would have obtained a similar result; whereas if, in the second experiment (*i.e.* the big one of 1919), his inoculations had been made from the blood of an infected man, instead of from infected horses and mules, the result might well have been different because of the exaltation in virulence of the strain from the human being, which might manifest itself as the result of passage through 2 human hosts consecutively without an intermediate passage through the invertebrate host" (Yorke, 1920). From this it is clear that Yorke at that time considered it possible for a single cyclical passage through tsetse to reduce "*T. rhodesiense*" to *T. brucei* again; whereas direct passage to another human host might enhance the power of the trypanosome to use man. A study of the records of the Uganda epidemic had led me to the same opinion about the effect of cyclical transmission (Duke, 1919).

The observations set forth in this paper and the experience acquired in the antelope investigation suggest to me that different strains of *T. brucei* differ in their power to overcome man's resistance. In other words, *T. brucei* is a potential parasite of man, in that it can in certain circumstances infect normal human beings. The latent powers of this trypanosome will not be called upon unless the game tsetses are compelled to feed extensively on man, in the absence of their normal food supply, the game. The majority of wild strains probably cannot touch man at all, and some strains are better able to infect him than others. Variations in man's resistance to *T. brucei* will, of course, play a part, but there is little doubt that the majority of wild strains are incapable of infecting even those whose trypanocidal power is lowest. The strains that infect man are at present called *T. rhodesiense*, though it is highly improbable that every *T. rhodesiense* can infect everybody. We have seen above that a trypanosome may lose the right to the title of *T. rhodesiense*, and there is some still unpublished evidence on this point which will appear in a later paper (Duke, 1935).

T. gambiense also possesses the power to infect man, but to a greater degree than *T. rhodesiense*; and we have seen that it can preserve this quality through innumerable animal passages over a period of at least 14 years. Moreover, in that particular instance the test was applied to a volunteer who had previously resisted a thorough exposure to a strain of *T. rhodesiense* whose power of infecting man, though impaired, was by no means completely lost. The

stability of the resistance of *T. gambiense* to human serum is also a striking feature of the *in vitro* experiments of Yorke and his colleagues.

Some years ago I discussed the origins and affinities of the three trypanosomes, *T. brucei*, *T. gambiense* and *T. rhodesiense*, and summed up in the following words:

"But the point that I wish to emphasise is that, in any strain of trypanosomes, the physiological characters—including ability to infect man—are largely determined by the environment. If the arguments here advanced are sound, then we are justified in concluding that the polymorphic mammalian trypanosomes of Africa all belong to a single species, and not a multitude of species....Included in this species there are many different varieties or strains, distinguishable from one another by characters of minor systematic importance. These strains are not immutable, but variable, and are determined by the environment in which the species lives: they are not constant, under varying external conditions, but each different strain is dependent upon, and is produced as a response to, a particular environment. If we call this species *Trypanosoma brucei*, then '*T. gambiense*,' '*T. rhodesiense*' and '*T. nigeriense*' are to be regarded as particular strains of *T. brucei* which have become, after sojourn in other hosts, more or less adapted to life in the blood of man" (Duke, 1921).

The fresh evidence seems to fit in with this view. So far, it is true, no one has yet witnessed the exhibition by an unequivocal *T. brucei* of the power to infect man, though it is of interest that Adams found strains of *T. brucei* recently isolated from game very much less sensitive to human serum *in vitro* than old laboratory strains (Adams, 1933). But the issue is confused by the convention whereby any *T. brucei*-like trypanosome recovered from game or from wild tsetse and proved capable of infecting man is, *ipso facto*, at once styled *T. rhodesiense* and assumed to have had previous acquaintance with man. *Strain* Tinde *I might easily be an example of this very phenomenon.* The hitherto unexplained instances of the infection of laboratory workers in Europe with species of trypanosomes supposedly non-pathogenic to man may also be examples of the exercise of the latent power of the *brucei* group to infect man, which has in some way been intensified by prolonged maintenance by direct passage.

That wild tsetse may in nature become infected from game with trypanosomes pathogenic to man can no longer be questioned, and some of these flies will doubtless be carrying strains that have at some time or other already lived in man's tissues. It is also obvious that the presence of cases of human trypanosomiasis, of whatever type, within the feeding range of game tsetses will greatly increase the likelihood of human strains, either pure or diluted by admixture with *T. brucei*, being found in both the game and fly of that area. Similarly, where several species of tsetse overlap, there will be found a mixture of the two main types of human trypanosomes such as occurs in Nigeria.

But I have for a long time believed that there are in nature strains of *T. brucei*, without any previous experience of man, that can, given the urge and the opportunity, use him as a host. It is of course possible that these human strains will only appear when an abnormally susceptible human subject is exposed to a specially endowed *T. brucei*. In the account of the experiments with antelope there is some recent evidence that natives do indeed differ in their susceptibility to *T. rhodesiense*. It is, however, quite certain now that a trypanosome can and does change in relation to a particular host species, *i.e.* man.

Taute's big experiment showed that 129 natives, including weaklings and diseased, completely withstood exposure to six different strains of *T. brucei*, presumably distributed one strain per man. This is a large sample of men and one specially selected to include susceptible units. The result seems to me to justify the conclusion that if man is to become infected by *T. brucei* the onus is on the trypanosome to overcome some degrees at least of the range of resistance possessed by a normal human community.

Partial resistance of a trypanosome to human serum, as demonstrated by the Liverpool *in vitro* technique and the use of a series of serum dilutions, does not, in my opinion, possess much practical significance as an indication of what a strain will do in man's blood. In nature a trypanosome will succeed or fail according to its ability to withstand the combined trypanocidal action of the tissues of the mammals on which the fly feeds, and in terms of resistance to human serum this, one is tempted to assume, means nothing less than undiluted serum, and possibly a good deal more.

Zimmermann (1931) examined the sera of human beings suffering from various diseases and found that, undiluted, all such sera retained a trypanolytic action against members of the polymorphic group, although this action was diminished in certain liver diseases. Fairbairn (1933) tested the trypanocidal action of twenty-nine human sera (European and native African) *in vitro* and found they were all of the same value. It is, however, highly probable that there are degrees of resistance in human beings and of susceptibility in different strains of trypanosomes that are not demonstrable in the test-tube.

The evidence acquired at Entebbe on this difficult subject is, as might be expected, not very extensive. On several occasions a volunteer who was exposed to and escaped cyclical infection with a strain of *T. rhodesiense* that had been for a long time in antelope, responded promptly to exposure to flies infective with strain LX. But the most significant observation is that a volunteer, who had previously resisted a thorough exposure to strain Tinde I, was subsequently bitten and infected by two flies carrying strain Tinde III. This is the only instance yet recorded where two strains of *T. rhodesiense*, both of which had resided for some time in antelope and been removed for months from contact with man, *behaved differently in the same individual human subject*. And it is interesting to note that this particular volunteer was unusually susceptible to infection with trypanosomes, for he was the

only one of six natives to yield to cyclical infection by *G. palpalis* infected with *T. rhodesiense* from antelope. These observations, which belong properly to a later report, are cited here because of their importance to the arguments now being considered. It is realised that this phenomenon is compatible also with the explanation that these two strains are "degenerating" *T. gambiense* that have lost touch with man to a different degree. So much of the evidence in this inquiry is indirect and also double-edged. But it seems to me probable that the stimulus of residence in man would have a more permanent restorative effect in the waning power of a *T. gambiense* than on the latent and possibly feeble infectivity to man of a strain of *T. brucei*. Had strain Tinde I been isolated from an antelope *in nature instead of at the laboratory* it would undoubtedly have been described as *T. brucei*. Adams' results in Uganda, in so far as they indicate that different strains possess different degrees of sensitiveness to human serum, confirm the view advanced here. The Liverpool work and Adams' independent publication also testify that the resistance of *T. gambiense* to human serum is a deeply rooted and enduring quality.

The investigations now in progress at the Institute show how complicated is the question of man's susceptibility to the *brucei* group. Unfortunately, it was not possible to carry out simultaneously with the tests on these volunteers an examination of their serum by the Liverpool method, and Corson's results with this method were not sufficiently encouraging to justify the abandonment of other lines of work calling urgently for completion. For my own part, I have acknowledged freely the scientific value of the researches of Yorke and his colleagues (Duke, 1933); but it seems to me that the novelty claimed for the hypothesis they advance is open to question, in so far as concerns the phylogeny and relations of the three principal members of the *brucei* group.

There is, of course, no longer any doubt that the genus *Homo* is highly resistant to all the trypanosomes of African game, including *T. brucei*. Neither is there any doubt that *T. brucei* is by nature first and foremost a parasite of game. But like other trypanosomes it can survive in species of vertebrates other than its normal mammalian hosts. Most of these are, however, of no real importance to the parasite in nature, and man in primitive Africa would fall into this category. His resistance to *T. brucei* though considerable is not absolute, and in *palpalis* regions he has paid the penalty for this imperfection by becoming the principal mammalian host for the representative of *T. brucei* in those regions, *T. gambiense*. Another common large mammal, which frequents the fly belts of Africa in troops and is a favoured food supply of the tsetse, the baboon, is possessed of natural resistance greater even than that of man, and is apparently completely inaccessible to all the game trypanosomes. But man in past ages in game-tsetse regions has lacked the biological incentive to adjust himself to tsetse-carried trypanosomes. Occasionally he falls to *T. brucei*, but when he does become infected, rapidly dies of what we now call *trypanosomiasis rhodesiensis*. Direct transmission from man to man may play a part in nature by assisting in the confirmation and spread of these

"human" strains, which are able to retain their power of infecting man through the vicissitudes of cyclical development in tsetse. But in primitive conditions the usual result would be the rapid or gradual elimination of man from contact with *T. brucei*, and the restoration of the game, trypanosome, game-tsetse régime. From time to time in the history of Africa, it may be that by the intervention of powerful disruptive forces such as war, rinderpest or famine, *T. brucei* has been constrained to depend temporarily on man for survival until normal conditions returned. At such times *T. rhodesiense* would appear. It needs no great effort of imagination to picture the now far distant origin of *T. gambiense*, in regions where the tsetse seek their food from man—not casually, but as their main source of mammalian blood.

Hitherto, comparatively few strains of *T. brucei* have been tested on man and exceedingly few in metacyclic form. Taute (1913) in his earlier experiments in Portuguese Nyasaland fed on himself *G. morsitans* carrying an antelope strain of this trypanosome and failed to induce infection. He then fed seventy-seven flies on five different antelope strains and when they became infective fed them on himself, with the same results. Later (1919) with Huber he carried out their famous inoculation experiments on human beings. Here he used six different strains from naturally infected horses and mules.

In judging the significance of these experiments it must be remembered that some importance probably attaches to the species of host from which the trypanosome is taken for the test on man. Evidence on this point has very recently been supplied by the investigations still in progress at Entebbe into the antelope reservoir. For example, on three separate occasions and with two different strains, a fly cyclically infected from an antelope that had been infected for many months with *T. rhodesiense* failed to infect volunteers, all of whom were subsequently proved to be susceptible to infection with other strains of *T. rhodesiense*. The blood of the same antelope was inoculated into monkeys by the syringe, and flies cyclically infected from these monkeys promptly infected volunteers. Here again, clear though these facts are, it must be remembered that any conclusions drawn therefrom are really based on the tacit assumption that all men are equal in their resistance to *T. brucei*.

The experiments of the present paper add three more "wild" *brucei* strains to the number properly tested on man, and still no wild strain has been convicted of successful assault. But we have at all events witnessed the reverse process, whereby *T. rhodesiense* reverts to *T. brucei*. And we have also seen how different from them both is *T. gambiense*, a trypanosome that for ages has depended on man for its survival in nature.

As matters stand at present, the claim of *T. rhodesiense* to distinction as a species, or even a subspecies, is indeed feeble. The name is, however, useful until it is decided whether *T. brucei* or *T. gambiense* can best absorb it zoologically. The evidence recently acquired at Entebbe seems to me to identify this trypanosome with *T. brucei*. If this be the correct interpretation, then the two types of human trypanosomiasis are to be ascribed to *T. gambiense*

and to *T. brucei* respectively. For the former trypanosome, by virtue of its long establishment as a parasite essentially dependent on man in primitive African conditions, surely possesses a fair claim at all events to "dominion status" if not to complete severance from its parent stock.

SUMMARY

1. A strain of *T. rhodesiense*, isolated from man and readily transmissible by tsetse, was passed by direct inoculation through a series of fourteen guinea-pigs over a period of 18 months. At the end of that time it had lost its transmissibility by *Glossina palpalis*, and it also failed to infect a volunteer.

2. Another line of the same strain, after 98 days in a bushbuck, 30 days in a fowl and 294 days in oxen, proved still readily transmissible by *G. palpalis* and also readily infective to man.

3. A second strain underwent seven consecutive cyclical passages through tsetse, then two passages by the syringe, and finally another cyclical passage, all save one in monkeys. When tested on man at the tenth and eleventh passages it was found to be non-infective.

4. A strain of *T. gambiense*, isolated in November 1920 from a patient from Fernando Po, was found in February 1934 to be readily infective to man. The strain was entirely non-transmissible by and almost completely non-infective to *G. palpalis*.

5. Three strains of *T. brucei*, one from the west, one from the north and one from the south of Uganda Protectorate, were found to be incapable of infecting normal healthy man. All the tests were carried out with cyclically infected tsetse.

6. A freshly isolated strain of *T. gambiense* from a Uganda native was transmitted to man by cyclically infected laboratory-bred *G. palpalis*.

7. A strain of *T. rhodesiense*, shortly after its recovery from a native of Tanganyika Territory, underwent three successive cyclical passages by laboratory-bred *G. palpalis* from monkey to monkey; at each passage the strain was tested on man and found to be readily infective.

8. A strain from Nigeria showing points of resemblance to both *T. gambiense* and *T. rhodesiense* was found to be pathogenic to man, on subcutaneous inoculation of infected blood, three years after its first isolation.

ACKNOWLEDGMENTS

I wish to acknowledge the assistance I have received from my colleagues, Dr J. M. Wallace and Mr J. S. McDonald, in the investigations recorded in this paper.

Postscriptum. Until after this paper had been written, an unfortunate oversight prevented me from seeing the publication by Dr H. M. O. Lester in vol. **27** of the *Annals of Tropical Medicine and Parasitology*. I decided, in spite of the many points of contact between Lester's work and my own, to make no alterations, in view of the appearance in the near future of a report on the antelope reservoir in which his valuable paper will be duly discussed.

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(*MS. received for publication* 14. v. 1934.—Ed.)

FURTHER STUDIES OF THE BEHAVIOUR OF *T. RHODESIENSE*, RECENTLY ISOLATED FROM MAN, IN ANTELOPE AND OTHER AFRICAN GAME ANIMALS

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The investigations described in this paper are the continuation of those published already (Duke, 1933). Their object is to determine the effect of prolonged residence in antelope on the trypanosomes of man.

There are two main aspects of this inquiry. It is necessary to study the ability of the trypanosome cyclically to infect tsetse, for if this power is lost the parasite cannot spread in nature; and it is also necessary to examine the power of the strain to infect man. Either or both these qualities might be affected by prolonged residence in antelope.

A brief review of previous work bearing on these points will be helpful, before proceeding to the experimental section.

At Mpumu in Uganda, Bruce and his collaborators infected several captive antelope, reedbuck, bushbuck and waterbuck, with *T. gambiense* (Bruce *et al.* 1911), and it fell to my lot to examine these animals from time to time up till 1912 when the laboratory at Mpumu was finally closed down.

The following were the conclusions reached in the final report on that investigation:

"(1) That antelope may remain capable of infecting *G. palpalis* with *T. gambiense* for a period of at least 22 months after their original infection with the trypanosome.

"(2) That there is some evidence to show that an antelope which has ceased to be infective for *T. gambiense* acquires some degree of immunity against re-infection" (Duke, 1912).

Actually in those experiments the latest evidence of infectivity to *G. palpalis* was a gland-infected fly that derived its infection from a bushbuck 13 months after the original infection of the antelope. Blood of the same bushbuck injected into a monkey 9 months later proved infective. It was noted that on two occasions strains isolated after long residence in antelope by subinoculation of citrated blood into clean monkeys, proved to be peculiarly well adapted to development in *G. palpalis*.

Weck, in 1914, inoculated an antelope with human blood containing *T. rhodesiense*. At the moment of writing no details are available about the subsequent history of this antelope, but attached to the review of the paper

in *Tropical Diseases Bulletin*, vol. **3**, there is a note stating that the animal already harboured a trypanosome before the inoculation of human blood.

Corson (1931) inoculated dik-dik with *T. rhodesiense* in order to determine whether trypanosomes could be demonstrated in the cerebro-spinal fluid of these animals. After some initial loss of condition the animals appeared not to suffer from their infection during the month they were kept under observation; but the author's objective called for their destruction before the effect of the infection could be finally determined.

Following on the work inaugurated by the League of Nations Commission in Uganda, *T. gambiense* and *T. rhodesiense* have both been studied in domestic ruminants. The latter trypanosome was found to be acutely pathogenic to both sheep and goats, but *T. gambiense* retained its transmissibility by tsetse for a period of over a year in sheep, without apparently harming its host.

In 1933 the first part of these investigations was published (Duke, 1933). The summary of that paper, in so far as it concerns antelope, reads as follows:

"1. A reedbuck, an ntalaganya (*Cephalopus caerulus melanorrheus*), 3 bushbuck, 1 oribi, 3 wild pigs (*Potamochoerus choeropotamus*) and one of an undetermined species and a serval cat have all been infected with strains of *T. rhodesiense* recently isolated from man.

"2. Infection in every instance was caused by the bite of cyclically infected laboratory-bred tsetse, either by *G. palpalis* or *G. morsitans*.

"3. The reedbuck and bushbuck and oribi showed no noteworthy symptoms ascribable to the trypanosome infection. The ntalaganya died of uncomplicated trypanosomiasis in 110 days.

"4. Clean laboratory-bred flies were infected from the reedbuck 107 days after infection; and from the ntalaganya 87 days."

In none of this earlier work was any test made on man himself. Corson's experience of *T. rhodesiense* maintained by direct passage in goats had suggested that this trypanosome would retain its power to infect man for a considerable time in ruminants. He found that a strain passaged by the syringe from goat to goat over a period of 19 months was at the end of that period still infective on direct inoculation into man (Corson, 1932). It was mainly for this reason that the long periods of infection of the antelope in the experiments of Part I of this research were allowed to elapse before any appeal was made to man.

In the work now to be described human volunteers have been freely used. An account of their enrolment has been given elsewhere (Duke, 1932 and 1934), and it is sufficient to record here that no untoward result occurred, and every one concerned has expressed himself as perfectly satisfied.

EXPERIMENTAL

The detailed history of the individual boxes of flies that go to make a transmissibility test is omitted, each test being presented in the form of a summary and all the tests on each strain grouped in a table for that strain.

In the tables and schedules + means that infection resulted, − means that the animal remained uninfected, and ± means that, of two men exposed, one did and one did not become infected.

In the schedules showing the history of the strains throughout this paper the date against an animal's designation is the day of its original infection. A thick line between two passage numbers means that the passage was made by cyclical transmission through *G. palpalis*; a thin line means direct transmission by the syringe. To ensure that flies placed on the volunteers did indeed feed, the tsetse were either starved for 48 hours before their meal on man and then given a second opportunity the next day, or they were first starved

Table I. *Transmissibility tests on animals infected with strain Tinde I*

Infecting animal	Period covered by test	Total flies dissected	Total infected flies	Infected flies %	Number of infected flies alive at 25th day: With glands infected	Number of infected flies alive at 25th day: Total	Transmissibility index	Results of feeding on clean animals: Animal before man	Man	Animal after man
Reedbuck I	13. ix. 32	472	39	8·2	4	26	*1·2*	+	.	.
,,	8–28. iii. 33	454	0	0	0	0	*0*	.	.	.
Oribi I	9–26. iii. 33	403	6	1·4	1	5	*0·3*	+	.	.
,,	28. xi.–8. xii. 33	395	2	0·5	0	0	*0*	.	.	.
Monkey 1188	3–8. x. 33	271	12	4·4	6	9	*2·9*	+	I − K −	.
,, 1212	5–15. xii. 33	234	7	3·1	3	3	*3·1*	+	V −	.
,, 1155	19–25. vii. 33	519	5	1·5	*2	2	*1·5*	+	D −	.
Reedbuck III	18–28. xi. 33	440	16	3·6	5	16	*1·1*	+	D and − K	.
Monkey 1174	26. ix.–2. x. 33	255	8	3·1	2	6	*1·0*	+	Q −	.
,, 1211	5–12. xii. 33	286	10	3·4	3	3	*3·4*	+	S and − X	.
,, 1143	2–12. vii. 33	322	12	3·7	1	5	*0·7*	+	.	.
,, 1153	22. vii.–2. viii. 33	487	22	4·5	11	15	*3·3*	+	D E F −	+
,, 1167	3–10. ix. 33	89	4	4·5	2	2	*4·5*	+	F −	.
,, 1168	3–22. ix. 33	422	10	2·3	2	5	*0·9*	+	F −	.
Oribi II	11–18. vii. 33	430	4	0·9	0	2	*0*	.	.	.

* These two flies showed very minute gland infections, mainly confined to the ducts and the adjacent anterior extremity of the body of the gland. In the second fly one gland was entirely devoid of flagellates, and in the other there was only a single small patch attached in one duct. This latter fly had failed to infect a guinea-pig before it bit man.

for 72 hours and then fed on man. *G. palpalis* feeds with avidity on man and, to make sure that exposure to infection has indeed taken place, any fly with an unfilled abdomen is killed by means of a hatpin, and removed from the box and dissected at once.

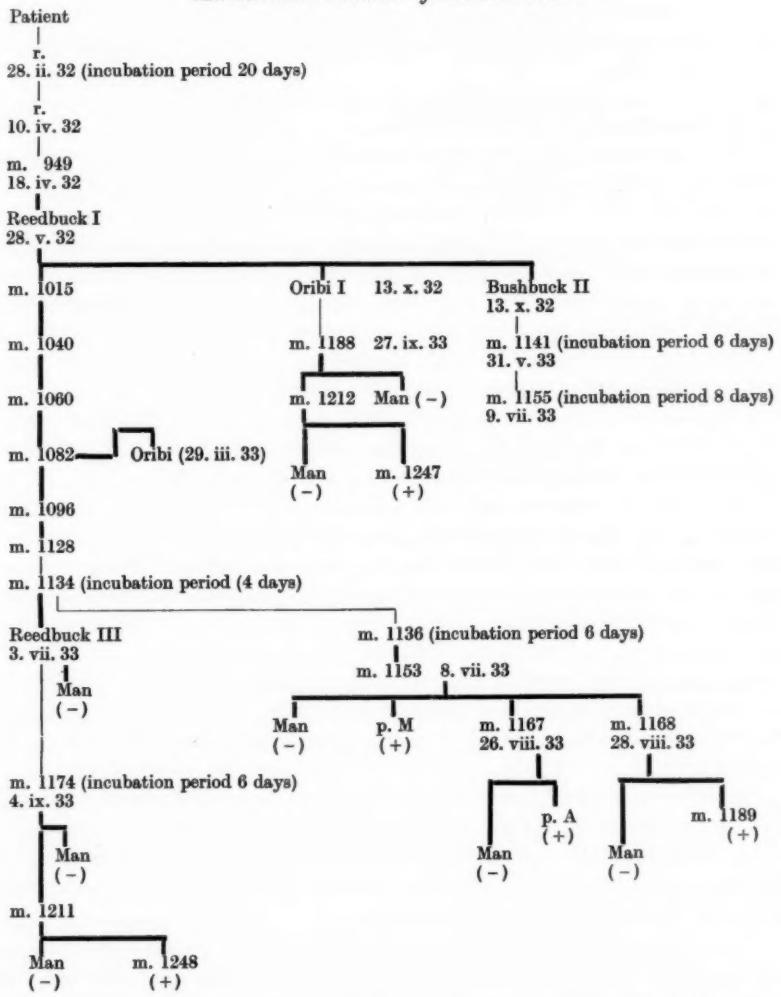
Strains Tinde I, II and III are those referred to in Part I of this investigation (Duke, 1933).

(1) *Strain Tinde I.* Isolated from a woman in northern Tanganyika Territory, who had been ill about 2 months.

The schedule and Table I show that this strain was on many occasions given the opportunity of infecting man and never succeeded, although nine different volunteers were exposed to infection. Taking now these tests one by one. The first on reedbuck I, performed about 100 days after the original infection of this antelope, is similar to the first test on bushbuck I (cf. strain

Tinde III below) in that only a small proportion of the infected 25-day old flies developed gland infections. But in contrast to the bushbuck figures, the

Maintenance schedule of strain Tinde I



second test on the reedbuck, some 280 days after the antelope's first infection, produced no infected flies. Apparently the infection with this strain has died out of this reedbuck, a conclusion confirmed by the following subinoculation experiments in each of which 10 c.c. of citrated antelope's blood were injected subcutaneously into clean monkeys:

Monkey	1094	received 10 c.c.	citrated blood of	reedbuck 18. iii. 33
„	1132	„	„	17. v. 33
„	1178	„	„	18. ix. 33
„	1223	„	„	17. xi. 33
„	1249	„	„	3. i. 34
„	1261	„	„	23. i. 34
„	1263	„	„	26. ii. 34

All these monkeys remained healthy.

The first test on oribi I was made some 150 days after the animal's original infection. In the second test, 265 days later and about 410 days after the first infection of the antelope, the transmissibility of the strain had undergone considerable reduction. In the test on monkey 1188 (inoculated from the oribi), the flies in one of the boxes were chloroformed and dissected on the 51st day after their infecting meal on the antelope. Three gland-infected flies were found. These had had access to a monkey on the 28th day, to man I on 30th, to man K on 33rd, to the monkey again on 34th day, to man K on the 37th and 38th days, to a second monkey on the 41st day and to man I on the 49th day. Only the second monkey became infected. Apparently the flies of this experiment were not infective on the 35th day after their first infecting meal. In any case, these flies were infective when they bit man for the last time.

The tests on monkeys 1211 and 1212 deserve special mention. The flies responsible for the infection of both these monkeys fed on but failed to infect man. The clean flies used for the tests on these two monkeys were nourished *entirely on clean human blood throughout their life in captivity*, until the time came to test them on clean animals. It was thought that nourishment of the flagellates on an exclusive diet of human blood might confer upon them the power to infect man; but in neither set of experiments was any effect apparent, the strain in each case failing to infect man. Three gland-infected flies were produced in each test, and all these six flies fed on volunteers without infecting them.

Reedbuck III was tested some 140 days after its original infection. Of the five gland-infected flies of this test, four fed on both volunteers and one on D only, and the glands of one fly were inoculated subcutaneously into man D. Neither volunteer became infected, although both men were proved subsequently to be sensitive to *T. rhodesiense*.

Both the gland-infected flies of the test on monkey 1174 fed well on volunteer Q, who did not become infected. Some months later this man was still perfectly healthy, and he was then given a dose of Bayer 205, in connection with an investigation of the prophylactic effect of this drug (Duke, 1934 *c*).

The tests on monkeys 1153, 1167 and 1168 have been described in the earlier report (Duke, 1935). That on monkey 1153 yielded eleven gland-infected flies. Volunteer D was bitten by eight of them, E by nine and F by six. None of these volunteers became infected, although all were subsequently

proved to be susceptible to infection with *T. rhodesiense*. These flies were readily infective to clean monkeys and guinea-pigs, both before and after feeding on man. Two flies of the test on monkey 1167 and one from monkey 1168 fed well on man F without infecting him. Both the flies of the test on monkey 1167 were full of human blood when dissected, and the glands of one of them injected subcutaneously into a guinea-pig infected the animal, the incubation period being 12 days.

The test on oribi II, applied some 114 days after its infection, shows that a reduction of transmissibility had also taken place in this animal. Taken in conjunction with the histories of the other three oribi employed in this investigation, it would appear that this species of antelope is not such a good host for *T. rhodesiense* as reedbuck and bushbuck.

Lastly, the test on monkey 1155 (carrying the trypanosome after it had resided for 102 days in reedbuck I, followed by 230 days in bushbuck II) shows also a reduction in transmissibility. The gland infections in these two flies were very slight indeed, though in one fly sufficient to infect a guinea-pig. Subinoculations of 10 c.c. citrated blood of bushbuck II into monkeys were made on 4. ix. 33 and 1. xii. 33, and on neither occasion did infection ensue. Apparently in this animal, as in reedbuck I, the infection is dying out.

From these records it is plain that this strain is non-pathogenic to man when carried cyclically by *G. palpalis*. The readiness with which flies carrying strain Tinde I infect guinea-pigs, monkeys and antelope, shows that the failure with man cannot be ascribed solely to the inadequacy of the inoculum introduced when flies carrying this strain bite man. Injection of the whole of both glands under the skin of the volunteer made no difference to the result. This strain was first isolated from man on 28. ii. 32 and first retested on man in September and October, 1933. That the ability to infect man has been lost is quite certain. But the strain may have owed its original establishment in man to a chance meeting with a hypersensitive individual, and this possibility must not be ignored. We shall return to this topic later on in the paper.

(2) *Strain Tinde II*. From a male 30 years of age from northern Tanganyika Territory.

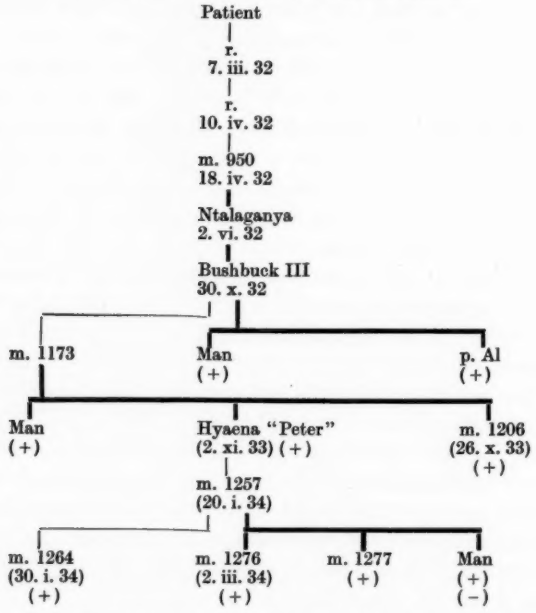
Table II. *Transmissibility tests on animals infected with strain Tinde II*

Infecting animal	Period covered by test	Total flies dissected	Total infected flies	Infected flies %	Number of infected flies alive at 25th day: With glands infected	Number of infected flies alive at 25th day: Total	Trans-missi-bility index	Results of feeding on clean animals: Animal before man	Results of feeding on clean animals: Man	Results of feeding on clean animals: Animal after man
Bushbuck III	29. viii.–16. ix. 33	616	11	1·7	5	5	*1·7*	–	H and – M	–
Monkey 1173	26. ix.–2. x. 33	349	4	1·1	2	3	*0·7*	+	R +	.
,, 1257	4–16. ii. 34	526	18	3·4	9	12	*2·5*	+	Y + W –	.
, 1264	6–17. ii. 34	457	11	2·4	2	3	*1·6*	+	.	.

The test on bushbuck III was carried out after the trypanosome had been in the antelope for some 10 months. The strain was then still readily trans-

missible by *Glossina*. Of the five gland-infected flies, all fed on man H and four fed on man M. In addition, the glands of one of the flies were inoculated subcutaneously into man M; a small tender swelling, which appeared at the site of inoculation, rapidly disappeared on the application of fomentations. Neither volunteer became infected. In a subsequent experiment 4 months later, man H was infected with *T. gambiense* and man M, after remaining healthy for several months, received a dose of Bayer 205 in connection with the investigations on the prophylactic effect of this drug.

Maintenance schedule of strain Tinde II



The test on monkey 1173 yielded two gland-infected flies, both of which fed on man R, who became infected after an incubation period of 8 days. Thus flies infected cyclically from the antelope failed to infect two volunteers, whereas flies infected from a monkey infected by syringe inoculation from this antelope promptly infected man. This phenomenon will be met with again in the account of strain Tinde III, where it will be more closely examined.

"Peter," a half-grown hyaena, was infected by the flies of this test, by subcutaneous inoculation of the glands of one fly. It is of considerable interest to record that at the time of writing, some 180 days after its infection, this engaging carnivore is in excellent health and spirits.

Monkey 1257 was inoculated with blood from the hyaena on 20. i. 34 and first showed trypanosomes in its peripheral blood 7 days later. Gland-infected flies infected from this monkey infected man Y, the incubation period being

8 days. The second volunteer, W, failed to become infected. The behaviour of this man calls for a detailed description in view of the discussion to follow later on. On 4. i. 34 he had been inoculated subcutaneously with the citrated blood of a guinea-pig heavily infected with strain Tinde III after it had been passed through fourteen guinea-pigs in series over a period of 18 months. On 1. ii. 34, his blood meantime remaining free from trypanosomes, he received a second inoculation of the same trypanosome, this time from a monkey—an experiment suggested by the behaviour in man of the trypanosomes of monkey 1173 above. On 8. iii. 34, the man being still in perfect health, 10 c.c. of his blood were inoculated into a clean monkey, which remained healthy.

The exposure of W to flies infected from monkey 1257 was as follows. On 13. iii. 34 a box of flies was put on him, the great majority feeding well. These flies were subsequently starved, and on 16. iii. 34 one died whose gut contained a pale green digest, and whose glands and gut were swarming with flagellates. The condition of its intestine showed that this fly had certainly not fed full on the volunteer, though it may of course have bitten him. On 14. iii. 34, more flies were put on him and then starved until 17. iii. 34, when all were killed and dissected. One fly, with a dark digest in its gut, was found to be heavily infected in both gut and glands. It is probable that this fly fed on the volunteer. Again, on 14. iii. 34 yet another box of flies was put on, from which on 19. iii. 34 two dead flies were removed and dissected. Both of these contained dark green digest and had swarming gland infections: it is improbable that either fed full on the volunteer, but it is very unlikely that neither of them bit him. All these flies before being placed on the volunteer W had been starved for the previous 48 hours. The two men Y and W were not bitten by the same flies, the boxes having been divided into two sections and one section being devoted to each man.

These later attempts to expose volunteer W to infection were thus singularly unfortunate. As the boxes were not destined for any further feedings after their contact with this volunteer, the usual precaution of removing flies with unfilled abdomina was not carried out. The rate of digestion in *G. palpalis* under the ordinary conditions of experiment is by now well known, and according to this criterion only one fly contained human blood digest. On the other hand, it is not unusual for a debilitated captive fly to bite several times without actually feeding, and many a time a fly that has been killed and removed as not having fed has been found on dissection to contain a small quantity of fresh red blood corpuscles. It was shown many years ago at Mpumu and again more recently by Corson that the mere introduction of the proboscis of a gland-infected fly will infect a *susceptible animal*. The question raised by the behaviour of volunteers D, W, K and L is, of course, whether there are in man degrees of susceptibility to certain strains of trypanosomes, and we shall return to this point later on.

It is highly probable that at least one gland-infected fly fed on volunteer W

and possible that others also bit him. If this is so there are four possible explanations of his escape from infection:

(1) That he possessed a natural resistance, above his fellows.

(2) That the flies did not bite him sufficiently thoroughly to infect him: this, in the case of one fly at all events, is highly improbable.

(3) That he was "protected" by the two previous inoculations of live trypanosomes.

(4) That individual infective flies differ in infecting power.

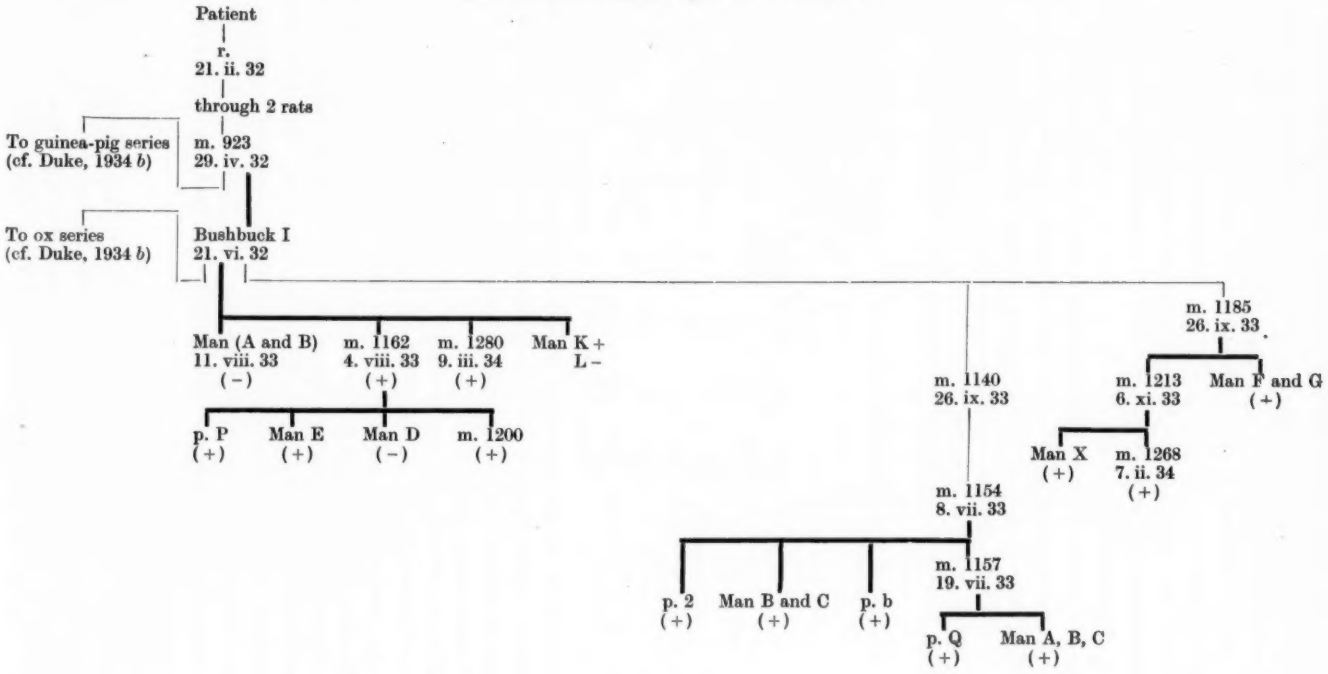
On 31. iii. 34 volunteer W received a subcutaneous inoculation of $\frac{3}{4}$ c.c. citrated blood of monkey 1277, an animal infected by the same flies that had failed to infect him. The site of the inoculation remained slightly tender and on the 8th day became painful and indurated; the man complained of general indisposition and his temperature ranged between normal and 100° F. On the 11th day his symptoms, local and general, had increased but no trypanosomes were detected in thick blood films examined at frequent intervals. On the 12th day trypanosomes were found for the first time. This experiment shows that volunteer W was in fact susceptible to the same phase of strain Tinde II as was carried by the flies that failed to infect him. Possibly his resistance was great enough to enable him to escape infection by fly-bite but not the direct inoculation of blood, the two modes of infection being in certain respects obviously different. Whether his previous two inoculations with living trypanosomes had enhanced his resistance cannot, unfortunately, be determined.

"Peter," the hyaena, showed no apparent ill effects from his infection with *T. rhodesiense*. Hitherto the only carnivora tested with this trypanosome are dogs, jackals and a serval cat (Duke, 1933), and all these died rapidly. Mr J. Carmichael, Assistant Veterinary Pathologist at Entebbe, found jackals very sensitive to *T. brucei* and to *T. congolense* (Carmichael, 1934). Game trypanosomes have been found in the wild hyaena in Uganda (Duke, 1913) and doubtless elsewhere, and this animal is widely distributed throughout the fly belts of Africa. So are the jackal and the serval cat, and all three animals are semi-diurnal in their habits. "Peter" came from a fly-free area, as did the jackals and the serval cat just mentioned. It is strange indeed to find the hyaena so indifferent and both the other animals so extremely sensitive to the trypanosomes of game. With the jackals, lack of acclimatisation may have played a part, but the serval cat had been kept as a pet for some months before its infection. It would be interesting to know how lions and leopards react to the *brucei* group, the domestic cat being extremely susceptible to *T. brucei*.

(3) *Strain Tinde III. T. rhodesiense*, from a male native aged 30 in northern Tanganyika Territory.

The schedule opposite shows maintenance of this strain and Table III the transmissibility tests carried out. These tests will now be considered in greater detail. To compare first of all the three tests wherein clean flies were fed upon the bushbuck. The records of individual experiments show that the dates of

Maintenance schedule of strain Tinde III



infection of the last gland-infected fly in each of the three tests were respectively the 130th, 429th and 594th days after the original infection of the bushbuck. The longest previous records of maintenance of cyclical transmissibility by man's trypanosomes in ruminants were obtained with *T. gambiense*. In a reedbuck this trypanosome remained capable of infecting the glands of *G. palpalis* for at least 13 months, and in a sheep for at least 15 months (Duke, 1912 and 1928).

In the two last tests on the bushbuck every infected fly that lived 25 days or longer developed a gland infection, whereas in the first test only two out of fifteen became infective. This suggests the gradual development of adaptation of the strain to the antelope. The detailed records of the last test show that all the infected flies were in boxes that had been put on the bushbuck between February 8th and 12th; whereas of 384 flies fed on the animal during

Table III. *Transmissibility tests on animals infected with strain Tinde III*

Infecting animal	Period covered by test	Total flies dissected	Total infected flies	Infected flies %	Number of infected flies alive at 25th day: With glands infected	Number of infected flies alive at 25th day: Total	Transmissibility index	Results of feeding on clean animals: Animal before man	Results of feeding on clean animals: Man	Results of feeding on clean animals: Animal after man
Bushbuck I	12–17. ix. 32	488	18	3·6	2	15	*0·4*	+	.	.
Monkey 1140	7–20. vii. 33	378	12	3·1	1	2	*1·5*	+	.	.
Bushbuck I	3–8. vii. 33	516	3	0·5	3	3	*0·6*	+	A and B −	+
Monkey 1154	17–20. vii. 33	152	4	2·6	1	2	*1·3*	+	B and C +	+
,, 1157	25–31. vii. 33	366	13	3·5	2	6	*1·1*	+	A, B, C	.
,, 1162	5–14. ix. 33	520	8	1·5	5	5	*1·5*	+	D −, E +	.
,, 1185	14–16. x. 33	454	16	3·5	9	10	*3·1*	+	F and G +	.
,, 1213	27. xii. 33–5. i. 34	169	6	3·5	1	2	*1·7*	+	X +	.
Bushbuck I	8–24. ii. 34	591	6	1·0	4	4	*1·0*	+	K +, L −	.

the remainder of the test, *i.e.* from February 14th to 24th, not a single one contained flagellates. This test thus affords an admirable illustration of a "negative phase" of the endogenous cycle of a trypanosome in the vertebrate host (Robertson, 1913).

We must turn now to the tests with monkeys 1154 and 1157 and the second bushbuck test, in all of which the same volunteers, A, B, and C, appear. At the time of these earlier tests on man the trypanosomes in monkeys 1154 and 1157 were regarded for all practical purposes as identical, although actually 1157 was infected by the syringe from 1154. At that time volunteers were scarce, and there was no reason to expect any difference between the behaviour of the trypanosome in the bushbuck itself and in monkeys inoculated from the antelope. The three available volunteers were accordingly devoted to what was regarded as a single test of *T. rhodesiense* that had resided for some 400 days in the bushbuck.

The actual details were as follows:

In the test on the bushbuck itself only two of the three gland-infected flies fed on man; one on volunteer A on 11. viii. 33 and the other on volunteer B on 13. viii. 33. The fly that bit volunteer A was killed and dissected 2 days later and was full of human blood; the other fly, 2 days after its feed on

man B, infected a clean animal. Each of these men was therefore bitten by one gland-infected fly, and in both flies the gland-infection was heavy.

In the test on monkey 1154 two gland-infected flies fed on man B and on man C; and in the test on monkey 1157, volunteers A and C were each fed on by three infective flies and volunteer B by one.

The dates on which each volunteer was bitten *for the last time* by an infective fly in each of the three tests and the dates on which symptoms were first noted or on which trypanosomes were first found in the blood (usually one and the same day) were as follows:

Test	Volunteer	Last bitten by flies	Trypanosomes in volunteer's blood
Second on bushbuck I	A	11. viii. 33	.
,, ,,	B	13. viii. 33	.
Monkey 1154 ,,	B	23. and 24. viii. 33	2. ix. 33
,, ,,	C	22. and 25. viii. 33	3. ix. 33
,, 1157	A	30. viii. 33 and 2. ix. 33	8. ix. 33
,, ,,	B	28. viii. 33	.
,, ,,	C	3. ix. 33 and 1. ix. 33	.

All the gland-infected flies were in different boxes, one infective fly to a box.

From the above time-table it is plain that man C was infected by the flies from 1154, his incubation period being 8–9 days.

It is equally plain that man A was infected by the flies of 1157 test, after an incubation period of 6 or more probably 9 days. Man B was almost certainly infected by the flies off m. 1154, with an incubation period of 10 days. To attribute the infection of A and B to the flies of the bushbuck test involves the assumption of an incubation period of 28 days for A and 20 days for B, and the regularity with which trypanosomes have appeared in all infected volunteers within at the most 12 days after exposure to infection (twenty-two of them have become infected up to the date of writing) makes this assumption both unnecessary and unjustifiable. Moreover, we have seen that strain Tinde II behaved in the same manner in bushbuck III. Thus volunteers A and B escaped infection when bitten by flies carrying trypanosomes derived directly from bushbuck I, but both these men and volunteer C all became infected when bitten by flies infected from the two monkeys 1154 and 1157, which in their turn had been infected from the bushbuck directly by the syringe.

The appearance of this phenomenon in two different strains and with two different antelope is remarkable. I am confident that the observations are above suspicion, although it is impossible at this stage to decide upon their interpretation. The stay in the monkey may have prepared the trypanosome against man's tissues. There is, however, another possible explanation, namely that in each subinoculation of the bushbuck's blood into a monkey the syringe has transmitted a form of trypanosome that would not have been taken up by the fly. It is not known yet whether infection by the syringe and by the cyclical method amount to exactly the same thing. My own view is that they do not. It is highly probable that flagellates which would die out in the fly's intestine, are delivered by the syringe into the subinoculated animal and the two methods of infection may, therefore, produce different results. If Robertson's

views on the endogenous cycle are correct then the great majority of the ordinary blood forms are doomed to disappear during the early stages of development in the tsetse.

Turning now to the third test on the bushbuck performed in February 1934, *i.e.* some 600 days after the animal's first infection, there is evidence that the two volunteers, K and L, used in this test, differed in their sensitiveness to this strain of *T. rhodesiense*. The test yielded four gland-infected flies, and two of them did not feed on man; the other two *fed well on both the volunteers*, and both these flies lived right through to the end of the experiment and infected a monkey before they bit man. Volunteer K developed a small tender indurated swelling at the site of the bites. According to the information supplied by the first test on this bushbuck, and also by the test on bushbuck III (strain Tinde II) above, it was to be expected that both these volunteers would escape. The fact that one did not means either that man K was hypersensitive or that his companion L was abnormally resistant. It is for the moment immaterial which of these alternatives is the truth; the important point is that the two men reacted differently to the same stimulus. The incubation period in volunteer K was 10 days. On 31. iii. 34, *i.e.* 17 days after his last exposure to the two flies of the bushbuck test, volunteer L received subcutaneously $1\frac{1}{2}$ c.c. citrated blood of monkey 1280 (the animal infected by the same flies that had failed to infect him) and trypanosomes appeared in his blood on the 6th day. The behaviour of these two men thus indicates strongly that differences in the resistance of individuals do exist and may therefore play a part in determining the infection of man with *T. rhodesiense* in nature. The infection of volunteer K was the first instance in the course of these investigations where a trypanosome after long residence in antelope was cyclically transmitted from this animal to man. As we have seen, on two other occasions cyclical transmission from antelope to man failed, whereas cyclical transmission of the same trypanosomes from monkeys—whether infected by inoculation of the antelope's blood or cyclically from the antelope—succeeded. The balance between infection and non-infection of man seems sometimes to be very finely adjusted. Actually, of six natives bitten by flies cyclically infected from bushbuck I and III, only one became infected—and this man had previously resisted a heavy exposure to the metacyclic forms of *T. rhodesiense* strain Tinde I. Yet the same strains taken up by the fly from monkeys infected all of eight volunteers tested.

We now come to the test on monkey 1162 (infected by the cyclical route from bushbuck I) and the volunteers D and E. The flies that infected this monkey took up their trypanosomes from the bushbuck on 3 and 5. vi. 33. The test is important and must be considered in detail. There were in all four flies with gland infections, three of which were heavy and one light. The last fly failed to infect a monkey which it bit on 26th and 28th day after the fly's first infecting feed. It was given access to volunteer D on the 31st day, but when dissected 2 days later contained only pale green digest; it may, therefore,

never have bitten the volunteer. The other three flies infected a guinea-pig on the 29th day and on the 31st day they fed well on volunteer E, in whose blood trypanosomes appeared 10 days later. These three flies had no opportunity of feeding on volunteer D, but the glands of one of them were injected subcutaneously without infecting him. The glands of another of the three were injected into and infected a white rat; and the glands of the last of the three were injected into man E, 6 days before trypanosomes appeared in his blood, and when he was, presumably, already infected.

The failure of man D to become infected was actually the first suggestion afforded by this research that individual human beings differ in their susceptibility to trypanosomes. Unfortunately, as we have seen, the only gland-infected fly that had access to him had a light gland infection and certainly did not take up blood, even if it bit him, which is itself doubtful. The fact, too, that this fly had previously failed to infect a monkey indicates that there was something unusual about it. The failure of the inoculation of the glands into man D though much more significant is not, however, conclusive, this method of infection being by no means infallible and possessing little value except when infection ensues. An interesting detail is that a small tender indurated swelling appeared on man D's arm at the site of the gland inoculation, disappearing in a day or so on treatment with fomentations. In view of what has already transpired we must be prepared to include volunteer D in the ranks of the probably resistant and admit that he perhaps owed his escape from the gland inoculation to his natural resistance. As already stated, this man was subsequently infected cyclically with *T. rhodesiense* strain LX, a strain that had been passed cyclically through *G. palpalis* for three generations from monkey to monkey immediately after its isolation from man; the incubation period of this strain in volunteer D was 10–11 days (Duke, 1935).

It will be useful at this juncture to summarise the observations on strain Tinde III in bushbuck I.

(*a*) The trypanosomes in this bushbuck were still capable of infecting the glands of *G. palpalis* 594 days after the animal's original infection. The gland infections were of the heavy kind characteristic of this strain.

(*b*) Two flies, each with a massive gland infection acquired from the bushbuck 401–404 days after its first infection, failed to infect man. Two volunteers were tested, and each fly bit one man.

(*c*) Monkey 1162 was infected by the two flies of the previous paragraph. Three flies with gland infections derived from monkey 1162 infected a volunteer. A second volunteer, possibly but not certainly exposed to infection by one of these flies, received subcutaneously the glands of another of them without becoming infected. The significance of this man's failure to become infected will be discussed later on.

(*d*) The blood of the bushbuck was inoculated into a monkey 344 days after the first infection of the antelope, and from this monkey the trypanosome was then passed by the syringe into two more monkeys. Flies infected from

these two monkeys infected three volunteers after incubation periods of 8, 9 and 10 days respectively. No local reaction where the flies fed on them was observed on the skin of any of these three men.

Thus flies infected from the bushbuck itself failed to infect man, whereas flies infected from monkeys that were infected from the bushbuck either by the cyclical method (m. 1162) or by the syringe (m. 1154, m. 1157 and m. 1167) infected man.

(*e*) Flies infected from the bushbuck some 600 days after its first infection infected one volunteer and failed to infect a second. This second volunteer proved susceptible to subcutaneous inoculation of citrated blood of a monkey infected by the same flies that failed to infect him.

The behaviour of these two men suggests strongly that prolonged residence in antelope had impaired the ability of this strain to infect man, and that the balance between infectivity and non-infectivity of this strain to man is very finely adjusted and susceptible to slight differences in the resistance of individual men.

(*f*) On every occasion when the blood of bushbuck I was inoculated into and infected a monkey, flies infected from that monkey infected man (five volunteers in all). Either, therefore, the species of mammal from which the flies acquire their cyclical infection influences the power of a strain to infect man, or there is a biological difference between direct inoculation of blood and cyclical infection.

It may be noted also that the test on monkey 1213 shows that a single fly is sufficient to infect man, so that there is no reason to ascribe the failure of the flies of the second bushbuck test to infect volunteers A and B to the inadequacy of the inoculum introduced.

(4) Experiments performed with reedbucks II and IV.

Reedbuck II. This animal, on its first arrival in February, 1933, as an unweaned kid, was set aside as a "control" in the antelope house. On August 24th, 1933, in the course of the routine examination of the four control antelope, it was found to be infected with trypanosomes. The strain on examination behaved like the strain of *T. rhodesiense* with which its companion, reedbuck III, had been infected, by tsetse, on July 5th, 1933. The resemblance was especially significant because the gland infections produced in *G. palpalis* by strain Tinde I were very characteristic, and flies infected from reedbuck II showed consistently the same scanty type of gland infection. The whole question of the infection of this animal has been dealt with in a previous publication (Duke, 1934*b*), the conclusion reached being that reedbuck II had been infected from reedbuck III by the agency of *Stomoxys* in the antelope enclosure.

Reedbuck IV. This young reedbuck was exposed on 15. viii. 33 to a fly whose heavy infection (gut and gland) was derived from bushbuck I. Trypanosomes were seen in the reedbuck's blood for the first time 8 days later, thick stained blood-films examined on the two previous days being negative. All

gland infections in clean flies infected from this reedbuck showed, however, the characteristic sparse infection with vacuolation of the flagellates constantly exhibited by strain Tinde I. No subinoculations were made from this reedbuck. The tests of the transmissibility of the trypanosomes in these two animals are shown in Table IV. Both these reedbuck are, therefore, to be regarded as carrying strain Tinde I, and reedbuck IV should in addition be infected with strain Tinde III. In both the first and the last tests of Table IV the volunteers were heavily exposed to infection, but all remained healthy; man U was subsequently infected with another strain of *T. rhodesiense* by fly-bite, and his companion eventually received a dose of Bayer 205 in the course of an investigation of the prophylactic action of that drug.

The following schedules show the history of the trypanosomes in these two animals, and Table IV the transmissibility tests carried out.

Maintenance schedules

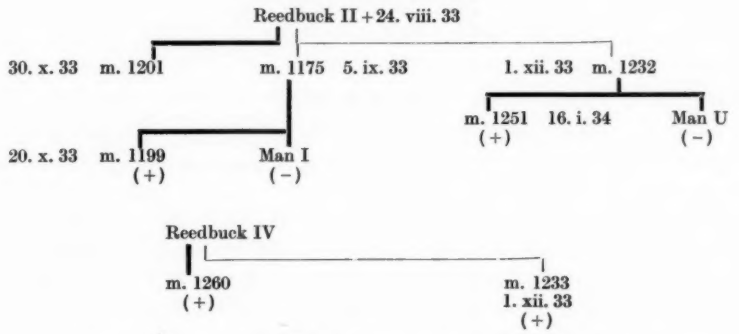


Table IV. *Transmissibility tests with the trypanosomes of reedbuck II and IV*

Infecting animal	Period covered by test	Total flies dissected	Total infected flies	Infected flies %	Number of infected flies alive at 25th day: With glands infected	Number of infected flies alive at 25th day: Total	Transmissibility index	Results of feeding on clean animals: Animal before man	Results of feeding on clean animals: Man	Results of feeding on clean animals: Animal after man
Reedbuck II	18–27. ix. 33	363	9	2·5	6	9	*1·6*	+	U –	.
Monkey 1175	17–24. ix. 33	383	4	1·0	1	2	*0·5*	+	I –	.
Reedbuck IV	29. xii. 33–10. i. 34	520	2	0·4	1	2	*0·2*	+	D –, K –	.
Monkey 1233	11–30. xii. 33	621	18	2·9	7	12	*1·7*	+	L –, Y –	.

(5) *Strain LI. T. gambiense*, isolated from an European on 28. ii. 33.

10 c.c. citrated blood of oribi III inoculated subcutaneously into a monkey on 26. ix. 33 failed to produce infection. It is hoped to re-examine this oribi at a later date.

The only other recent attempt at infecting an antelope with *T. gambiense* was the exposure of an adult female situtunga to strain L from a Uganda native. Two sheep exposed to the same flies that subsequently bit this antelope became infected.

Maintenance schedule of T. gambiense, strain LI

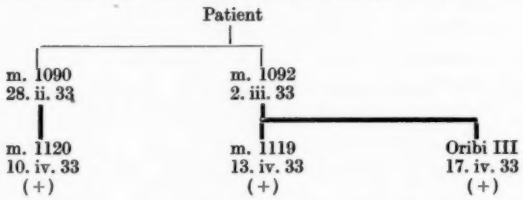


Table V. *Transmissibility tests on animals infected with strain LI.*

Infecting animal	Period covered by test	Total flies dissected	Total infected flies	Infected flies %	Number of infected flies alive at 25th day: With glands infected	Number of infected flies alive at 25th day: Total	Transmissibility test
m. 1090–1092	15–31. iii. 33	775	12	1·2	8	10	*1·2*
Oribi III	30. v.–17. vi. 33	332	4	1·2	1	1	*1·2*

Discussion

Perhaps the most interesting and at the same time unexpected of the observations recorded above is the difference between the infectivity to man of flies cyclically infected from monkeys and from the bushbuck respectively, all the flies carrying the same strain at approximately the same stage in its laboratory career. With both strains Tinde II and III all the tests performed on monkeys that had been infected by the inoculation of bushbuck's blood yielded gland-infected flies that were readily infective to man. In these tests all the six volunteers exposed to infective flies became infected, whereas of six volunteers exposed to flies cyclically infected from the bushbuck, only one became infected.

In a single test on a monkey infected cyclically from a bushbuck (m. 1162, strain Tinde III), the flies infected one volunteer, whereas a second volunteer inoculated subcutaneously with both the glands of one of these flies did not become infected. This experiment lies midway between cyclical infection from antelope to man and cyclical passage to man from a monkey which had been infected by direct inoculation from the antelope. There is ample evidence in this and the previous paper (Duke, 1935) to show that cyclical passage, *per se*, does not necessarily exert any demonstrable effect on the power to infect man; but the behaviour of the trypanosome in monkey 1162 suggests that the method of infection, direct or cyclical, may indeed influence this character where it is not very strongly developed.

It will be noted that where a strain was isolated from a bushbuck by direct inoculation of its blood into monkeys, and then passaged by the cyclical method from monkey to monkey, it consistently retained the power of infecting man (cf. schedule of strain Tinde III). Strain Tinde III retained this power

after residence in a bushbuck for 594 days. Strain Tinde III was also still readily infective to *G. palpalis* 594 days after its introduction into bushbuck I.

The behaviour of strain Tinde I shows, on the other hand, that *T. rhodesiense*, even when maintained in monkeys, may lose altogether its power of infecting man. This strain even failed to infect volunteer K, who, there is a reason to believe, was abnormally sensitive to trypanosome infection. It is of course possible that the native from whom strain Tinde I was originally isolated was also an abnormally sensitive individual. This strain was also less successful in adjusting itself to antelope, reedbuck I having thrown off all signs of infection within a year.

These observations show what may happen to *T. rhodesiense* when it is introduced into antelope and removed from all contact with man.

They also show that long residence in antelope may impair the power of a trypanosome to infect man. Certain species of antelope are admirable hosts of the trypanosome known as *T. rhodesiense*; they suffer no apparent inconvenience from the prolonged presence of the trypanosome in their blood, and the protozoan preserves well its transmissibility by tsetse. But as a reservoir from which tsetse can become infected with trypanosomes pathogenic to man the observations recorded in this paper suggest that these animals do not constitute so great a menace as has hitherto been supposed.

Variations in man's resistance to trypanosomes

During this research three significant indications were noted that individual volunteers differ in their natural resistance to *T. rhodesiense*, and all three instances occurred in experiments carried out with strains that had resided for months in non-primates (antelope or hyaena). Volunteer D in the test on monkey 1162 (infected by fly from bushbuck I), volunteer L in the third test on bushbuck I, and volunteer W in the test on monkey 1257 (infected from the hyaena) all appeared to be possessed of an abnormally high resistance. Of these examples the best substantiated is volunteer L, in comparison to his fellow, K. In none of these volunteers was the resistance absolute: D subsequently responded to cyclical infection with another strain, and L and W to direct inoculation of the same trypanosomes that had previously failed to infect them by the cyclical method. The differences between individuals in their resistance to trypanosomes are therefore in all probability very slight, so small that they would probably not be discernible in tests carried out with serum *in vitro*, although Lester reports an instance of subnormal resistance in a secondary syphilitic revealed by this test (Lester, 1933). In nature this factor would only affect the career of strains that had been long in game and whose infectivity to man is low: it would have no effect on strains like LX. We may suppose therefore that long residence of *T. rhodesiense* in game animals so tends to impair its power of infecting man that its metacyclic forms can only infect people of abnormally low resistance and perhaps not even these. It may well be that in the course of time circulation in the blood of game

would eliminate this power altogether. But at all events in the earlier stages of its dissociation from man the trypanosome may recover its infectivity to him by brief residence in other hosts such as the monkey—another, if a lower, primate, which differs from man by being far more susceptible to trypanosomes.

Virulence and method of infection

On the subject of virulence Lester points out that cyclical transmission of a strain may or may not be attended by change in this character. He has already shown that acquired resistance to human serum can survive this process (Lester, 1932). It is known, also, that resistance to drugs survives unchanged, and similarly with the quality of transmissibility by tsetse (Duke, 1934 *a*); and to these may now be added pathogenicity to man. Cyclical transmission is evidently not such a "melting pot" as was once generally supposed; and, when all is said, virulence is a notoriously variable quality and difficult to standardise and estimate.

Lester also discusses the possibility that the number of trypanosomes injected may affect virulence where the cyclical method is employed, and suggests that if many gland-infected flies bite an animal a more virulent infection may result than when only one or two participate. He mentions Lloyd's and Paisley's calculation that less than 1000 metacyclic forms of *T. vivax* and *T. congolense* will be injected by a fly while it feeds. Rodhain (1913) estimated that a single *G. morsitans* infective with *T. brucei* inoculates at its bite a minimum of 1562 flagellates. Oehler's work on infection with a single trypanosome also bears on this point (Oehler, 1913); he found nothing noteworthy about the duration of an infection starting from a single parasite. My own experience supplies the following observations: a single gland-infected fly can infect man; there is no apparent difference in the course of infections in monkeys or guinea-pigs when one or when several infective flies are used to infect; flies carrying strain Tinde I readily infect monkeys and guinea-pigs although their glands contain sometimes extraordinarily few and never very many flagellates, yet on one occasion five and on another ten gland-infected flies carrying this strain failed to infect a volunteer; and finally, the effect of subcutaneous inoculation of the whole of both glands of an infective fly, both with strain Tinde I and the other strains, differs in no appreciable way from that following the bite of a single fly. Though not of course decisive, these considerations suggest that where the metacyclic method is employed, and provided the flies feed properly, the number of infected flies used does not make an appreciable difference either to the immediate result or to the subsequent course of the disease. Moreover, in nature, it is highly unlikely that exposure of man to cyclically infected wild flies, in numbers similar to those obtained by Taylor by the use of the incubator, will ever occur, and certainly not on any one occasion. The issue will depend on single bites or at the most on very small numbers of infective flies.

The injection of infected blood is, however, a very different matter. As already stated, I do not believe that the two methods are essentially equivalent. The forms of trypanosome in the blood may, for all we know, differ in their attributes from the metacyclic forms in the fly, and in addition there is the factor of protein shock and the ensuing local and general reaction. The one is the natural and the other a wholly unnatural method of spreading a trypanosome.

This point is, I think, a good deal more important than a perusal of the literature on trypanosome research would suggest. Writing on malaria in a review of the subject in the *Tropical Diseases Bulletin*, **23**, 331, Clayton Lane remarks:

"Although Ley compares an injection [*i.e.* of blood containing the organisms of malaria] to an immense mosquito bite, there is much to indicate that this is by no means the case; that these two methods are indeed sufficiently distinct to necessitate caution in transferring inductions, made upon injected, to natural cases....Clearly infections acquired as sporozoits react in this respect differently to those received as trophozoits, and there would seem to be no justification for assuming, without evidence, that inductions from infected can be transferred to natural cases. The two classes should then be separately considered."

No biologist can compare the local reaction following a successful injection of infected blood with that occasionally produced by the bite of an infective fly, without a more or less acute sense of shame and disgust at the clumsiness and crudity of his handiwork. Out of seventeen volunteers infected at Entebbe by fly-bite, five showed a small area of tender swelling where a fly bit them. The other twelve showed no local symptoms at all. One of the five was volunteer K, and two of the remaining four were infected with strain LX. Whether native hunters ever become infected by the direct method during their wolfish dissection of their prey, has not yet been determined. Scraps of raw flesh are often devoured on the spot in the excitement of the moment, and infection through the skin is certainly possible, covered as the men usually are from head to foot with gore. But even if direct infection in this manner does occur, there is still no question that the trypanosomes we are discussing depend on development in tsetse for their spread in nature.

The origin of T. rhodesiense

On this question these experiments afford some indirect evidence. Lester in his latest paper (1933), supported by a wide experience of human trypanosomiasis under a variety of conditions in Nigeria, joins Kleine in believing *T. rhodesiense* to be a virulent variety of *T. gambiense*. He allows that on rare occasions *T. brucei* may infect man, but deems this explanation inadequate to explain the general incidence of *T. rhodesiense*. This view offers at once a reasonable explanation of the varieties of human trypanosomes occurring in Nigeria and possibly elsewhere where *T. gambiense* is common and

man comes in contact with tsetses that feed on game. It involves the assumption that *T. gambiense*, under natural conditions of spread, can increase in virulence and assume all the characters of *T. brucei*, while retaining its power to infect man.

Starting from their removal by the fly from man's blood, we can picture the career of different strains of *T. rhodesiense* living in the antelope of a game tsetse area. Some strains strongly, some weakly pathogenic to man; frequent admixture with *T. brucei*, innocuous to man; reinforcement, now and then, by strains recently in contact with man; but always subjected to the influence of the antelope tissues and entirely reliant on cyclical transmission to reach a new host. After a year or two away from man there will perhaps be in the wild fly in such an area still some strains that can still infect man. But the evidence points to a fairly rapid elimination of elements pathogenic to man (unless reinforced by admixture or by a spell in human blood), for the transmission is by tsetse which derive their food and their infection from the game.

The alternative explanation of *T. rhodesiense* is that certain strains of *T. brucei* are pathogenic to man, possibly only to individuals of subnormal resistance, perhaps also to normal man. If this be so there is no need to invoke the intervention of *T. gambiense*, for *T. rhodesiense* is admittedly indistinguishable from *T. brucei* save one only in the one respect of pathogenicity to man.

The evidence before us has a bearing on this alternative explanation in that it reveals the possibility that there are differences between individual Africans in their resistance to trypanosomes, although these differences may only assert their influence within a very narrow range of strain-infectivity to man. It also shows how a strain may change in relation to a certain species of host and how easily *T. rhodesiense* may become non-pathogenic to man. There is also the significant fact that the same individual may resist one strain and succumb to another (volunteer K resisted strain Tinde I and fell to strain Tinde III), which shows that these two strains differed in their behaviour towards one and the same individual. On the other hand, the effect of prolonged residence in antelope on the three strains examined tells against the probability that a strain weakly pathogenic to man would retain the faculty for long in an uninhabitated fly-belt.

I have pointed out that comparatively few strains of wild *T. brucei* from uninhabitated areas have so far been tested in man, and until this defect is remedied we cannot claim to know much about this trypanosome. There are in the wild fly of Uganda strains of *T. congolense* that cannot infect carnivora or primates. These strains were once called *T. nanum*, but their right to independence has been questioned in recent years. Has *T. rhodesiense* any greater right than "*T. nanum*" to specific isolation? *T. nanum* has been "made" pathogenic to monkeys and *T. vivax* to rabbits. *T. vivax* has even been reported in man (without suffering change of name), and Kleine in a private communication mentions that he has on one occasion seen a freshly isolated

T. vivax in the blood of a monkey, trypanosomes being present for a single day only. Why not credit *T. brucei* with a similar versatility in its dealings with man? It cannot be denied that "*T. nanum*" occurs in nature in wild tsetse in Uganda (Duke, 1916), and although it is unlikely that carnivora or monkeys are essential to *T. congolense*, nevertheless hyaenas and jackals are at least as common and as useful to this trypanosome as man is to *T. brucei* in game-tsetse areas. The accepted explanation of the differences between *T. nanum* and *T. congolense* may equally well apply to *T. rhodesiense* and *T. brucei*.

My own views on this problem are unchanged by the experience of recent months. I still favour the close relationship of *T. rhodesiense* to *T. brucei*, and consider that *T. gambiense*, phylogenetically, is *T. brucei* adjusted in past ages to man.

Kleine's work with the League of Nations Commission has shown that typical *T. rhodesiense* soon learns to adapt itself to man, and in so doing assumes more and more the guise of *T. gambiense*. This view will therefore account for the conglomeration of strains of both types in *gambiense* areas where man comes in contact with *T. brucei* and with the game tsetses.

The debate is still open, and it behoves those interested in the speedy solution of these questions to turn resolutely to the acquisition of fresh evidence to fill the gaps in our knowledge revealed by a review of the progress made in recent years.

There is one obvious line of research, open to all those able to command an adequate supply of clean game tsetse, that promises information of the utmost value; and that is the study of the effect of a game-tsetse game environment on a typical *T. gambiense*. At Entebbe the only antelope infected with a transmissible strain of *T. gambiense* (oribi III, strain LI) shows signs of having shaken off its infection within a comparatively short time. This experiment will be continued later on, and an attempt will be made on my return from leave to ensure a supply of *G. morsitans* from Tanganyika Territory.

The experience acquired at Mpumu shows that with suitable strains of *T. gambiense* there should be no great difficulty, provided of course that casual infection with either *T. brucei* or *T. rhodesiense* can be excluded.

It may be that mere change of tsetse species will in course of time suffice to produce the metamorphosis of *T. gambiense* into *T. rhodesiense*. But it seems to me far more likely that residence in antelope will be found to be also an essential part of the process.

The question demanding answer is: Does *T. gambiense*, when transferred from its normal environment to a complete dependence upon game and game tsetse, assume the characters of *T. brucei*? There may be other points awaiting settlement in this rapidly contracting field of research, but surely none possesses equal importance or promises a more definite outcome.

SUMMARY

1. Evidence is produced to show that *T. rhodesiense* may retain its cyclical transmissibility by *Glossina* for at least 600 days in an antelope.

2. Prolonged residence in these animals tends to impair the power of a strain to infect man, when infection is attempted by the cyclical route. Thus of six volunteers exposed to cyclically infected tsetse carrying *T. rhodesiense* that had been for many months continuously in antelope, only one became infected. *All these flies derived their infections from the antelope itself.* As the cyclical method is the one operating in nature, these observations suggest that although certain species of antelope are admirable hosts for *T. rhodesiense*, yet as a reservoir from which tsetse can become infected with trypanosomes pathogenic to man these animals do not constitute so great a menace as has hitherto been supposed.

3. In contrast to (2), in every instance where the trypanosomes from the antelope, before being tested on man, were inoculated by the syringe into a monkey, every volunteer exposed became infected.

In the single experiment where the monkey was infected by tsetse instead of by direct inoculation from the antelope, one volunteer became infected and another did not. There is thus a suggestion that the passage through the monkey prepared the trypanosome for survival in man. On the other hand, the behaviour of strain Tinde I shows that *T. rhodesiense* may lose its power to infect man in spite of repeated passage through monkeys.

4. Some further indirect evidence is produced to show that *T. rhodesiense* may owe its origin to *T. brucei*, and that pathogenicity to man may be a property possessed in different degrees by different strains of *T. brucei* in nature.

5. These researches suggest, also, that human beings differ in their susceptibility to *T. rhodesiense*, but that these differences only operate within a narrow range of variation in the power of strains to infect man. In other words, strains strongly pathogenic to man will infect anyone, but strains whose power has been weakened will only be able to use individuals of subnormal resistance.

6. Two different strains of *T. rhodesiense*, have been shown to behave differently in the same man. One did and the other did not infect him.

7. There may be a difference in the suitability of the various species of antelope to act as hosts to *T. rhodesiense*. Thus the bushbuck seems to be a better host to the trypanosome than the oribi.

8. A young hyaena infected with *T. rhodesiense* for 180 days remained in excellent health. Flies infected from a monkey sub-inoculated with the blood of this hyaena infected a volunteer: the trypanosome had then been 80 days in the hyaena.

9. Clean flies that had taken their first two meals off monkeys infected with a strain of *T. rhodesiense* non-pathogenic to man, were nourished entirely on human blood during the first 3 weeks of the cycle of development of the

trypanosomes in their interior. These flies, on the completion of this cycle, were still unable to infect man.

10. A strain of *T. rhodesiense*, after a series of cyclical passages through a reedbuck and six monkeys, was found to be non-pathogenic to man. The possibility has to be borne in mind that this strain owed its original association with man to meeting an abnormally susceptible individual. This strain since its arrival at the Institute has been tested on nine different volunteers, and none of them became infected. There is therefore nothing to distinguish it from *T. brucei*, save the fact of its isolation from man.

11. It is recorded that a single cyclically infected fly infected a volunteer at a single feed.

12. It is shown that the appearance of a tender indurated swelling at the site of the bite of an infective fly is a not uncommon symptom of an infection with *T. rhodesiense*. Local tenderness and swelling were also noted where the glands of an infected fly had been inoculated subcutaneously, even in cases where no infection ensued. Where inoculations of blood were employed on man the local disturbance disappeared rapidly when no infection resulted, but when infection took place the local symptoms steadily increased during the last few days of the incubation period, and before trypanosomes were recognised in the peripheral blood.

13. The only infection of an antelope with *T. gambiense* carried out during this research was that of oribi III with strain LI. This strain at its first isolation was not very transmissible by *G. palpalis*, and after a few months in the antelope showed signs of failure to adjust itself of this particular host. Another strain, mentioned in a previous paper (Duke, 1935), failed to infect an adult female situtunga, although two sheep exposed to the same strain at the same time duly became infected.

ACKNOWLEDGMENTS

In addition to much help received from my colleagues Dr J. M. Wallace and Mr J. S. MacDonald, I wish to acknowledge the valuable assistance rendered by Dr J. J. Black, in whose hands rested the original recruiting and the treatment of the volunteers used in this research.

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(*MS. received for publication* 18. v. 1934.—Ed.)

STUDIES ON THE LIFE HISTORY OF *PANOPISTUS PRICEI* SINITSIN, 1931 [TREMATODA]

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(With Plate I containing Figs. 1–7)

CONTENTS

	PAGE
Introduction	93
Technique used in the study of larval stages	93
Description of life-history stages	94
Sporocyst	94
Cercaria	94
Metacercaria	95
Information concerning experimental animals	97
Intermediate hosts	97
Source of parasite eggs used in experiments	97
Infection experiments with *Panopistus pricei*	98
Summary	100
References	100
Explanation of Plate I	100

Introduction

Sinitsin (1931) described a fluke, *Panopistus pricei*, from the small intestine of the shrew, *Blarina brevicauda*, and assumed on the basis of morphological similarity that a metacercaria which he collected from the pericardium of the snail *Gastrodonta suppressa* was that of this species. A consideration of Sinitsin's evidence, especially of his brief description of the metacercaria, in connection with the findings of the writer, does not seem to substantiate Sinitsin's assumption.

In this paper the life history of *Panopistus pricei*, as determined experimentally, is described; the snail *Zonitoides arboreus* (Say) was used as the first and second intermediate host. This paper supplements a previous report by the writer (Krull, 1934).

Technique used in the study of larval stages

Most of the cercariae used for study were washed off the bodies of infected snails with a pipette having a fine opening. By this method only those cercariae which had completed their growth were obtained, these being comparable to cercariae shed by aquatic snails. A few cercariae used for verifying anatomical details were secured by dissection, as these usually contained less refractive material.

Larvae to be studied in the living condition were usually mounted in egg albumen. This technique has already been described by the writer (Krull, 1934).

Description of life-history stages

Sporocyst. Sporocyst (Fig. 1) a branching tubular structure varying considerably in its extent and number of branches.

Several individual sporocysts have been recovered from a single snail; they were comparatively easy to free from the host tissue. The sporocysts have been recovered from the digestive gland, the gonad, and tissues surrounding the latter organ. Movements of sporocysts are sluggish. The method of escape of the cercariae from the sporocysts has not been determined.

Cercaria. Body small; tail very short, indistinctly separated from body. Cuticula provided with spines distributed as follows: Rather long spines at anterior tip of body diminishing in size and number posteriorly, extending posteriad, both dorsally and laterally, to level of mid-oral region of sucker, and on ventral side scattered spines usually continuing to level of posterior end of acetabulum; surfaces of oral sucker and acetabulum covered uniformly with posteriorly directed, short, rather thick spines; posterior end of tail with a few, very fine spines; large broad spines on surface of oral sucker, and larger sensory spines on body and acetabulum as shown in Fig. 2; of the large sensory spines, the lateral pair at posterior end of body are apparently the largest, the spinous portion of these sometimes 3μ long; sensory spines very similar to those on the cercaria of *Brachylaemus virginiana* as described by Krull (1934). Oral sucker larger than acetabulum; both very muscular; anterior border of acetabulum approximately equatorial. Prepharynx very short; pharynx large and muscular; oesophagus short and thick, bifurcating and forming short, broad caeca extending laterad and posteriad to level of aceta-

Table I. *Measurements of cercariae and organs under different conditions.*

			Living specimens (5) while under pressure	Specimens (11) killed and mounted in 10 % formalin	Specimens (6) stained and mounted
Body length, including tail		Maximum	346	248	240
		Minimum	256	178	174
Body width		Maximum	150	98	70
		Minimum	115	78	53
Oral sucker	Length	Maximum	86	60	60
		Minimum	69	50	50
	Width	Maximum	84	53	55
		Minimum	74	43	45
Pharynx	Length	Maximum	32	28	—
		Minimum	22	21	—
	Width	Maximum	36	22	18
		Minimum	30	17	17
Acetabulum	Length	Maximum	73	52	42
		Minimum	58	34	48
	Width	Maximum	78	55	54
		Minimum	61	50	46

bulum. Three pairs of gland cells situated at level of posterior end of acetabulum, with fine ducts running anteriorly, these ducts traceable to border of oral sucker, converging here and apparently extending to border of mouth opening. Excretory bladder small, at posterior end of body, receiving a pair of collecting tubules extending anteriad to level of pharynx, then turning and extending posteriad, these descending branches being at least partially ciliated. Bladder discharging to exterior through a pair of ducts extending through tail, terminal parts of these ducts thickened and projecting beyond cuticula. Flame cells, seven pairs; four pairs preacetabular and three pairs postacetabular. Germinal mass rather well defined, situated anterior to excretory bladder.

Measurements of cercariae (in μ) are given in Table I.

The specimens upon which this description is based were washed off the bodies of infected snails serving as first intermediate hosts. The sensory spines of the cercaria were very difficult to see in spite of their large size; it is possible that there is an additional pair of spines at the anterior end of the body on the dorsal side and a couple of spines lateral to the acetabulum, but after repeated trials the presence of this pair of spines could not be verified. Neutral red, janus green and brilliant cresyl blue were helpful in studying the anatomy of the cercaria mounted in egg albumen; with the latter stain the digestive system was purple, the germinal mass green and the remaining structures blue. The fine gland cell ducts could be traced dorsal to and beyond the oral sucker, but the openings were not observed; these ducts are discussed further in the description of the metacercaria. The germinal mass near the posterior end of the cercaria body in contracted specimens is transversely elongated, but in elongated specimens the long axis of the mass is parallel to the long axis of the body. This mass gives rise in the metacercaria to accessory parts of male and female reproductive systems. It is questionable whether the testes and ovary arise from it, but, if so, only a very few cells of the mass are involved. Sinitsin (1931) in a much more detailed study of the genital primordia involving the larval stages of *Plagioporus*, apparently *P. siliculus* Sinitsin and *P. virens* Sinitsin, stated that in the flukes the "Anlage" from which the ovary, "albumin gland" and Laurer's canal originate arises independently of the "Anlage" from which the rest of the genitalia develop.

Metacercaria. Body varying considerably in shape with state of contraction as indicated in Figs. 5 and 6. Cuticula 8μ thick in living specimens and 3–4μ thick in stained and mounted specimens relaxed in water prior to fixation. Cuticula devoid of spines, except for remnants of some of the larger, sensory cercaria spines, containing very fine globules similar to the ones more abundant in the subcuticular layers. Suckers large, oral sucker slightly larger than acetabulum, the latter equatorial in position. Prepharynx not discernible except in extended living specimens. Pharynx large and muscular, anterior end slightly incised. Oesophagus short, thick-walled and muscular, bifurcating and forming caeca having a more or less serpentine course to near posterior

end of body, undulations much more pronounced in contracted specimens. In living specimens under pressure, walls of caeca 2μ thick; in contracted, stained and mounted specimens, average width of caeca 28μ; in specimens relaxed in water and stained and mounted, average width of caeca 22μ, and width of lumina 4–10μ. Remnants persisting of cercaria gland cells, the ducts usually quite prominent but exceedingly fine. Bladder small, with muscular walls, at posterior end of body, receiving a pair of ducts extending anteriad, lateral to caecum on their respective sides, to level of pharynx, turning and extending posteriad to level of ovary; these descending branches are ciliated. Each descending branch bifurcating at level of ovary and forming branches, one continuing posteriad and the other turning and continuing anteriad to beyond acetabulum. Bladder discharging through a short duct opening at excretory pore at posterior end of body. Testes irregularly oval or ovate, anterior testis slightly larger than posterior, both larger than ovary; ratio of size of anterior testis to that of ovary, approximately 6 : 4. In contracted specimens, anterior testis to left of median line, postacetabular and slightly more anterior than ovary, posterior testis to right of median line, usually bordering on it. Ovary immediately in front of posterior testis. In relaxed specimens, sex organs tandem and somewhat oblique to long axis of body, anterior testis submedian and posterior testis slightly to right of median line. Vasa efferentia uniting between testes and forming vas deferens extending to genital pore. Ovary in relaxed specimens in field of and posterior to anterior testis. Oviduct broad, opening dorsally, after uniting with a short postero-dorsally directed Laurer's canal, receiving broad common vitelline duct formed by union of right and left vitelline ducts, the latter broad and conspicuous in metacercaria. Oötype surrounded by a large developing Mehlis' gland, emerging as the uterus with first a transverse course, then a posterior course to genital pore as indicated in Fig. 7. Genital pore in contracted and relaxed specimens in median line, ventral and at level of, or slightly posterior to, ends of caeca. Numerous cells, originating in the genital mass of the cercaria, surrounding the ducts except the vitelline ducts, of the sex organs, the most pronounced concentrations being in the regions of Mehlis' gland and genital pore.

Ducts from the three pairs of gland cells posterior to acetabulum, extending anteriorly, converging somewhat at pharynx, and continuing anteriorly along pharynx. The ducts could be traced only for a short distance anterior to the pharynx, and it is the opinion of the writer that they discharge at the mouth opening in the oral sucker.

In the development of the metacercaria the cercaria tail is severed from the body in much the same way as that described for a related form, *Brachylaemus virginiana*, as described by Krull (1934). The tail is usually lost when the larva is intermediate in size between the cercaria and the metacercaria. In numerous cases, however, the tail is retained and held by a fragment after the larva is fully grown; in one such larva the tail vestige was spherical and 22μ in diameter.

Table II. *Measurements of metacercariae and organs.*

			Series A	Series B	Series C	Series D
Body length		Maximum	755	884	750	545
		Minimum	535	772	585	484
Body width		Maximum	448	310	300	340
		Minimum	345	256	235	280
Oral sucker	Length	Maximum	195	174	140	137
		Minimum	156	140	120	110
	Width	Maximum	188	164	132	136
		Minimum	160	147	124	116
Pharynx	Length	Maximum	88	90	—	—
		Minimum	67	73	—	—
	Width	Maximum	88	82	65	60
		Minimum	78	73	55	58
Acetabulum	Length	Maximum	182	174	130	113
		Minimum	133	148	110	112
	Width	Maximum	214	180	130	125
		Minimum	160	160	112	124
Anterior testis— mean diameter		Maximum	70	72	—	—
		Minimum	54	48	—	—
Posterior testis— mean diameter		Maximum	62	68	—	—
		Minimum	48	52	—	—
Ovary— mean diameter		Maximum	50	52	—	—
		Minimum	34	36	—	—

The measurements of the metacercaria (in μ) are given in Table II: Series A representing the measurements of seven specimens killed in corrosive acetic fixative, then stained and mounted; series B those of seven specimens relaxed in water for 12 hours, after which they were killed in corrosive acetic and stained and mounted; series C those of two living specimens under pressure in egg albumen 25 hours; and series D those of two specimens mounted in albumen 5 hours.

Information concerning experimental animals

Intermediate hosts. Zonitoides arboreus (identified by Mr Wm. B. Marshall of the U.S. National Museum), the snails used in the experiments, were the second generation of laboratory-raised snails, the original stock having been collected in the vicinity of Beltsville, Maryland. The snails were raised in terraria containing sterilised material, this precaution being taken in order to kill all snails and parasite eggs of extraneous origin. Snails used for infection experiments were transferred from terraria, retained for stocks of snails, to fingerbowl terraria. While the conditions under which the snails were raised precluded any possibility of extraneous infections, nevertheless thirty-six were examined and found to be negative for trematode infestations. All snails were fed on rolled oats and lettuce, the former being used as a conveyor for the infective parasite eggs.

Source of parasite eggs used in experiments. The parasite eggs used in the infection experiments were recovered from a single specimen of *Panopistus pricei* collected from the intestine of a shrew, *Blarina brevicauda*, which was taken in the vicinity of Beltsville, Maryland. The parasite was preserved for identification.

Infection experiments with *Panopistus pricei*

Experiments involving intermediate hosts. Since only some of the more important experiments are described in detail, certain general facts concerning the infection of the first and second intermediate hosts will be given.

The first intermediate host, *Zonitoides arboreus*, apparently becomes infected by eating the parasite eggs. The sporocysts which produce cercariae were situated in the gonad and digestive gland, several sporocysts having been recovered from a single snail. Snails which were actively shedding cercariae were covered by them, as many as 257 cercariae having been washed off of one of these small infected snails at one time. The second intermediate host becomes infected, apparently, by coming in contact with a snail, a first intermediate host, which is actively shedding cercariae. After the cercariae have been transferred to the second intermediate host snail, the cercariae gain entrance to the kidney where they grow and transform into metacercariae. If only a few larvae are present, the metacercariae usually migrate to the pericardial cavity after they have completed their growth in the kidney. Here they remain attached to the pericardium by the suckers and apparently remain relatively inactive. If many larvae are present in the second intermediate host, the growth of some larvae is retarded, and some of the metacercariae after they have completed their growth migrate to the pericardium while the majority remain in the kidney. Metacercariae do not encyst, and those in the kidney, apparently, always have at least a small amount of kidney material in the caeca, while the metacercariae in the pericardial cavity seem always to have empty caeca. The position of metacercariae in the host was determined by dissection and serial sections of entire snails. The largest number of larvae recovered from a second intermediate host was forty, and the largest number of metacercariae was fifteen. First intermediate host snails having a sporocyst infection will not serve as second intermediate hosts. The following experiments give more specific information concerning the infecting of snails with *Panopistus pricei.*

Exp. No. 1. Nine young fully grown *Zonitoides arboreus* were subjected to infection in their terrarium November 13th, 1933. Four of the nine snails became infected; cercariae were observed on the surface of two of the four snails 44 days after being subjected to infection, and cercariae were observed on the four infected snails 7 days later. One of these four infected snails was alive and still shedding cercariae April 14th, 1934.

Exp. No. 2. Nine young fully grown *Zonitoides arboreus* were subjected to infection in their terrarium November 13th, 1933, and four of these snails became infected. This and the preceding experiment establish *Zonitoides arboreus* as a first intermediate host of *Panopistus pricei.*

Exp. No. 3. An infected snail having numerous cercariae on its surface was transferred January 3rd, 1934, to a terrarium containing 11 uninfected adult *Zonitoides arboreus.* The infected snail was removed January 24th, 1934,

at which time thirty-four cercariae were washed off the snail, indicating that it was still actively shedding. One of the snails in the terrarium was examined by dissection January 24th, 1934, and forty larvae were recovered, ten being metacercariae and the others cercariae of various sizes. Another snail examined February 19th, 1934, contained seventeen larvae of metacercaria proportions, although three had loosely attached tails. Two snails were examined the following day; from one of these snails twenty-one larvae were recovered, all of which were of metacercaria proportions, although there was considerable variation in size and five had loosely attached tails. Some of the larvae which were confined to the kidney, had food particles in the caeca, while those in the pericardial cavity were without food. In the other snail twenty-one larvae were observed; two of these had tails which were well differentiated from the body, although completely attached, these being typical cercariae showing considerable growth. The distribution of the larvae in the body was the same as in the previous snail. This experiment establishes *Zonitoides arboreus* as not only a first, but also a second intermediate host of *Panopistus pricei*.

Exp. No. 4. An uninfected snail was transferred with an infected snail, which was actively shedding cercariae, to a covered watch glass, Bureau of Plant Industry model, and left for 24 hours. The snail which had been subjected to the infection was examined 33 days later, and two fully-grown metacercariae, having no food particles in the caeca, were recovered from the pericardial cavity.

Exp. No. 5. Ten snails were transferred with an infected snail taken from Exp. 2 and actively shedding cercariae, to a small covered stender dish and left for 1 hour, after which they were again separated. One of the snails which had been subjected to infection was examined a week later and two cercariae were recovered from the kidney. Both of the cercariae had grown considerably. The caeca which had grown and now reached the genital mass were full of food, and the tail was very distinct and well differentiated from the cercaria body. Another snail was examined 2 weeks after it had been subjected to infection and from it a single fully-grown metacercaria was recovered. Of the remaining snails, examined 3 weeks after they had been subjected to infection, four were negative, three had two, five and one metacercariae, respectively, in the pericardial cavity, and the remaining snail had one large cercaria in the kidney; in all of these metacercariae the caeca were empty.

Exps. 3, 4 and 5 show that: (1) The cercariae are transferred, apparently, to the second intermediate host by contact; (2) the metacercariae may complete their growth in 2 weeks, and practically all complete their growth in 3 weeks; (3) the cercariae after being transferred to the second intermediate host gain entrance to the kidney where they grow and transform into metacercariae while living on the kidney tissue; and (4) when growth of the metacercaria has been completed the caeca may be empty and the metacercariae may enter the pericardial cavity.

On account of the difficulty involved in keeping shrews in captivity no

attempt was made to carry on experiments with them. Laboratory-raised white-footed mice, *Peromyscus leucopus noveboracensis*, could not be infected with this parasite and have never been found naturally infected.

Summary

The life history of *Panopistus pricei*, as determined experimentally, has been described, together with the life history stages, including the sporocyst, cercaria and metacercaria. The snail *Zonitoides arboreus* has been determined experimentally to be a first and second intermediate host of the parasite.

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EXPLANATION OF PLATE I

Panopistus pricei

Fig. 1. Sporocyst, stained and mounted specimen.

Fig. 2. Cercaria, ventral view.

Fig. 3. Cercaria, stained and mounted specimen, ventral view.

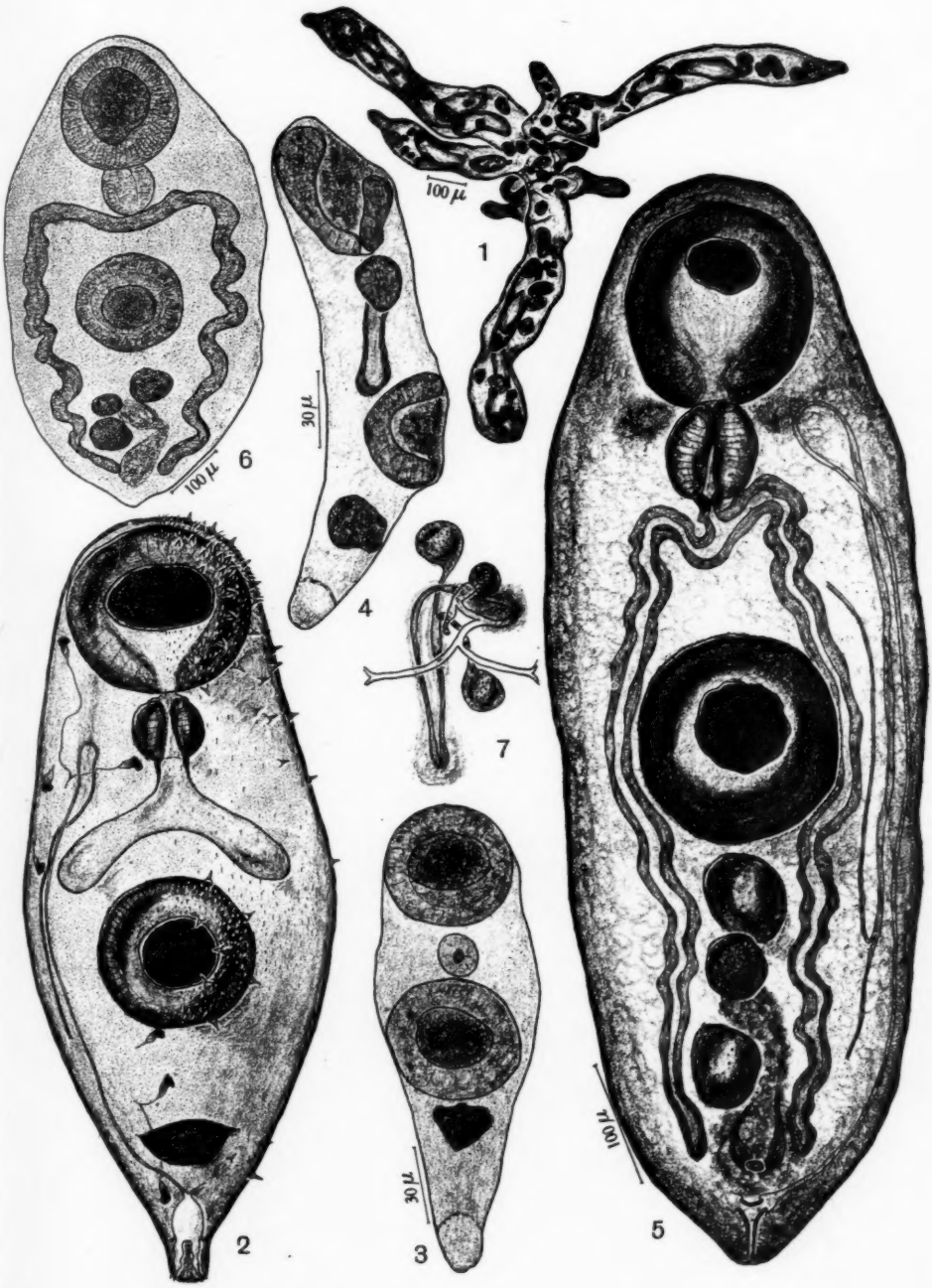
Fig. 4. Cercaria, stained and mounted specimen, lateral view.

Fig. 5. Metacercaria, stained and mounted after being relaxed in water, ventral view. Excretory vessels supplied from living material.

Fig. 6. Metacercaria, stained and mounted specimen killed under slight pressure without being relaxed in water, ventral view. This and the metacercaria represented in Fig. 5 were taken from the same snail.

Fig. 7. Genitalia, dorsal view

(*MS. received for publication* 22. v. 1934.—Ed.)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EPIBRACHIELLA IMPUDICA (NORDMANN) (COPEPODA)

By W. HAROLD LEIGH-SHARPE, M.Sc. (Lond.)

(With 8 Figures in the Text)

Material and habitat

Two adult females bearing males, on the gills of *Trigla cuculus* at Plymouth, collected by Miss Miriam Rothschild in November 1933.

Record

An animal, both sexes of which were originally described by Nordmann (1832) under the name of *Brachiella impudica*, has been the subject of considerable controversy, and has been discussed by no one better than by L. Harrison Matthews (1923), whom I am in some measure able to confirm. But no previous authors have described the bulla; and they all state that the number of posterior processes is six, having overlooked the small ventral pair, except Brian (1906), who, without giving any description, figures one process of this pair. Bassett-Smith (1896, 1899) unjustifiably removed this species to the genus *Thysanote*, having no male specimens. Wilson (1915), having no material but judging from Nordmann's figures, correctly stated that this species could not belong to the genus *Thysanote*, and at the same time pointed out that the male was so different from that of *Brachiella* as to warrant its removal from that genus. Accordingly he erected for it the genus *Epibrachiella*. Matthews (1923) was the first investigator after Nordmann to discover males, and the main object of his paper was to correct Nordmann's description of the males on two points, amending Wilson's generic diagnosis of the male. Both Wilson and Matthews suppose the female animal so badly figured by Scott and Scott (1913) under the name of *Thysanote impudica* to be *Epibrachiella impudica*. I have examined the eighteen specimens at the British Museum from the British coasts, deposited by Scott and Scott, and they all resemble Nordmann's and Brian's figures and are not in the least like Scott's own figure, the largest specimens having a bulla, and all of them a process on each second maxilla (Fig. 3).

But perhaps the greatest point of controversy is the presence or absence in the female of this process on the outside of each second maxilla as long as or longer than the rest of the arm. These processes were figured by Nordmann and Brian, but not found by Bassett-Smith, Matthews, or myself on specimens taken at Plymouth, so that Bassett-Smith was induced, on account of their small size and the absence of these processes, to call his Plymouth specimens variety *parva*. I think, however, that his specimens, which can be seen in

the British Museum, are young forms. As stated above, Scott's specimens have these processes, but he has not figured them.

Matthews also draws attention to what he calls another variation, namely, the tendency of the dorsal pair of posterior processes to fuse, as in Fig. 1 B. I again suggest that this is dependent on the age of the specimen, for one of my adult females is as in Fig. 1 B, and the other as in Fig. 4. Since Brian and Nordmann figure their females as in Fig. 4, I presume this is the more common and therefore older form.

Description

Female (*Plymouth specimens*)

Body. Total length 5·1 mm.

Head 1 mm., inclined at an angle to the neck.

Neck curved anteriorly, length 1·4 mm., with a small spherical knob on the dorsal side. The specimen in the Norman Coll. Brit. Mus. has a strongly chitinised head and neck, straight as in Brian's figure.

Trunk. Strongly flattened dorsiventrally without pits or grooves, length 1·2 mm., width 2·25 mm., with bilobed embossed shoulders laterally (one apparently figured by Brian), with four pairs of posterior processes, the postero-lateral corners each provided with two moderately short processes of about equal length, another pair of longer submedian appendages springing from the dorsal aspect, and a little in front of the posterior margin, and a very small submedian ventral pair (Fig. 1 A). Genital process robust, vulvae prominent with chitinous rims (one female showing two spermatophores, and the other female one, Fig. 5). Eggstrings ovate, short, robust, 2·1 mm. in length, longer than the posterior processes. The specimen in the Norman Coll. Brit. Mus. has elongated eggstrings and of a different shape.

The **appendages** (Fig. 6) are:

Antennules four-articled, the terminal article the longest ending in three short spines in different planes.

Antennae large and inflated, bluntly rounded at the extremities which face each other across the mouth cone; they bear a small exopodite consisting of a small papilla ending in a spine.

First maxillae of the usual type consisting of an unsegmented endopodite which is bipartite at the distal end, and an exopodite which is tipped by two straight spines.

Second maxillae short, abruptly flexed across the base of the neck (Fig. 1 A), resembling an elbow joint, and in these young specimens they appear to be fused together. The bulla is pateriform.

Maxillipedes are clawed appendages consisting of a slender basal article, provided on the inner side with the usual two cushions of spines between which is a small downwardly curved spine (*Sp*). The terminal article is slender and terminates in a long curved claw with an accessory claw at its base.

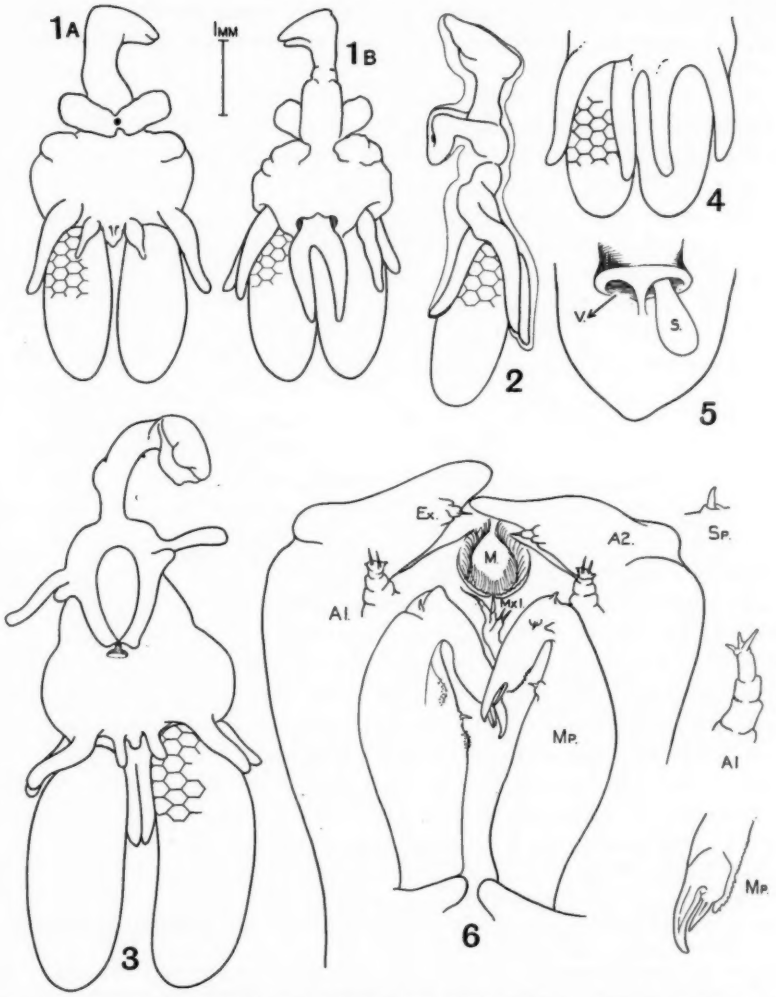

Fig. 1. *Epibrachiella impudica*, ♀, one of the author's adult specimens from Plymouth. A, in ventral aspect; B, in dorsal aspect.

Fig. 2. *Epibrachiella impudica*, ♀, the same animal as in Fig. 1 in left lateral aspect.

Fig. 3. *Epibrachiella impudica*, ♀, one of Scott and Scott's specimens from the British coasts deposited at the British Museum and considered by them to be *Thysanote*, in ventral aspect, drawn to same scale as Figs. 1 and 2.

Fig. 4. *Epibrachiella impudica*, ♀, the posterior end of the author's other adult specimen from Plymouth in dorsal aspect.

Fig. 5. *Epibrachiella impudica*, ♀, enlarged view of the genital process. *V*, vulva; *S*, spermatophore.

Fig. 6. *Epibrachiella impudica*, ♀, enlarged view of anterior end of the specimen in Fig. 1 in ventral aspect. *A* 1, antennule; *A* 2, antenna; *Ex*, exopodite; *M*, mouth; *Mx* 1, first maxilla; *Mp*, maxillipede; *Sp*, spine on maxillipede.

Proximal to the claw the portion of the article facing the distal cushion of spines is serrated. Two spines are present at the proximal end of the article.

Male (*Plymouth specimens*)

The general outline of the male can be seen from Fig. 7. That the waist is not so constricted as in Matthews' Fig. 1 is probably due to pressure on the

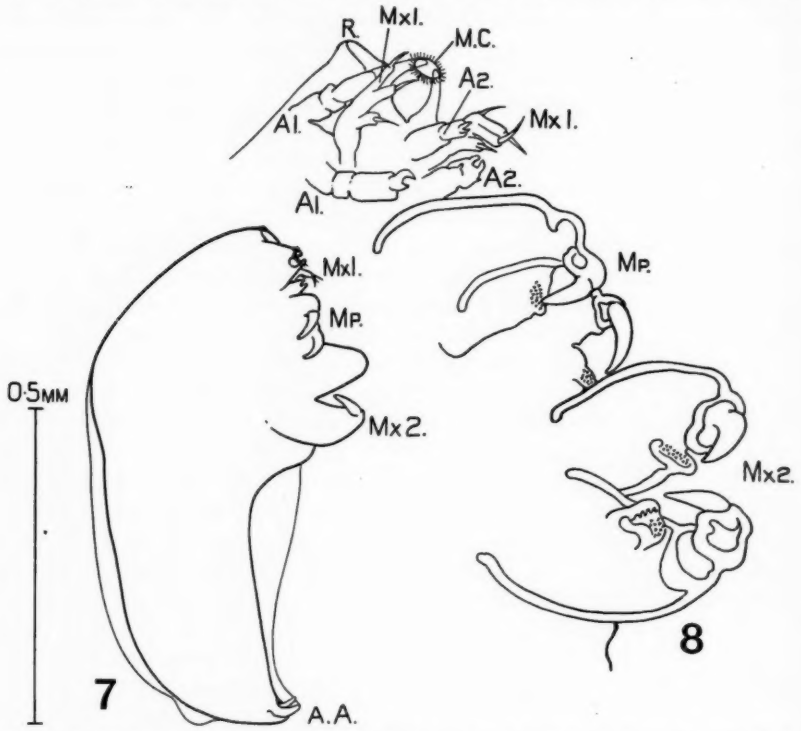

Fig. 7. *Epibrachiella impudica*, ♂, in right lateral aspect. *A.A.* abdominal appendages; *Mx*2, second maxillae. Other lettering as in Fig. 6.

Fig. 8. *Epibrachiella impudica*, ♂, enlarged view of the anterior appendages *in situ*. *M.C.* mouth cone; *R*, rostrum; *Mx*2, second maxillae. Other lettering as in Fig. 6.

coverslip. The males on Scott and Scott's Brit. Mus. specimens have this narrow waist and the general inflated appearance of the trunk which shows no indication of segmentation. The head and trunk are of about equal size, in the same straight line in the Plymouth specimens; in Scott and Scott's specimens the head is carried at an angle, and the trunk is greatly curved forward. The rostrum is not two-pointed as Nordmann considers, with which Matthews' description agrees. The abdomen is not distinguishable from the trunk and bears two moderately large abdominal appendages.

The paired appendages may be seen *in situ* in Fig. 8.

The *first antennae* (*A* 1) are four-articled, terminating in a spine, the proximal article is the largest, and the middle one the smallest.

The *second antennae* (*A* 2) are biramose, the exopodite with two articles, the distal of which is bifid. I am unable to make out the mandible, but according to Matthews it bears seven large triangular backwardly-directed teeth, with a small secondary tooth posterior to each of the first three.

The *first maxillae* (*Mx* 1) are of the usual tripartite type. The bipartite endopodite, and the smaller exopodite each ends in an elongated curved spine.

The *second maxillae* (*Mx* 2) are large and stout, and bear a stout strongly curved claw, the apex of which shuts down into a socket in the basal part elaborately supported by chitin and having denticles at its base.

The *maxillipedes* (*Mp*) resemble the second maxillae in being stout and bearing a claw which is opposed against the outer edge of the base which is denticulated.

The mouth cone is prominent.

There is no dorsal carapace.

The appendages of the males of Scott and Scott's specimens in the Brit. Mus. are exactly similar to the above.

NOTE ON GENERIC DIAGNOSIS

It is remarkable that Wilson removed the species under discussion from the genus *Brachiella* to the genus *Epibrachiella* by reason of characters most of which are now demonstrated to be erroneous, *viz.* the supposed absence of a genital process in the female, and a number of features in the male including the supposed segmentation of the trunk, and two-pointed rostrum. Judging by the male alone, I fail to see any reasons for removing this species from the genus *Brachiella*. However, the presence of eight posterior processes (only six figured by all authors except Brian), and to a less extent, the presence of anterior shoulders on the trunk in the female, is, in my opinion, sufficient to warrant its removal from the genus *Brachiella*, and therefore *Epibrachiella* should stand as a valid genus by priority although originally founded under several misapprehensions.

NOTE ON SPECIFIC VARIATION

It is not suggested that the hypothesis that Group I (the Plymouth specimens of Bassett-Smith, Matthews, and myself) are young forms of Group II (the specimens of Scott and Scott, Brian, and Nordmann) has been proved. It is based on the facts that in the female:

(1) The specimens of Group I are definitely smaller (see Figs. 2 and 3 to same scale).

(2) Variability in the dorsal posterior processes of two specimens from the identical *Trigla* (*T. gurnardus* in the case of Matthews, *T. cuculus* in the case of myself) (Figs. 1B and 4).

(3) Other characters being the same, the egg-strings of the specimen in the Norman collection are elongated and of a different shape, and its cephalothorax is not bent at an angle and has a funnel-like formation as in Brian's figure.

Some of Scott and Scott's large specimens are also in this condition; the smaller their specimens the more the cephalothorax is curved anteriorly.

Opposed to this view is the fact that in the forms of Group I the second maxillae have an elbow-bend, and appear to me to be entirely fused. In Group II the second maxillae are outspread and widely separate as far as the bulla. If Group I is the younger it is to be expected that the converse would be the case.

However, in a collection of *Epibrachiella* made by Dr C. L. Oakley from *Trigla* sp. in the neighbourhood of Plymouth there are representatives of both stages, some with processes on the second maxillae, and some without, but with elongated arms unfused (as in Fig. 3), forming an interesting connecting link.

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(*MS. received for publication* 2. VI. 1934.—Ed.)

SOME NEW AND RARE LERNAEIDAE (COPEPODA) FROM PLYMOUTH

By W. HAROLD LEIGH-SHARPE, M.Sc. (Lond.)

(With 2 Figures in the Text)

On 19. v. 1934, while trawling from S.S. *Salpa*, kindly placed at my disposal by the Marine Biological Association, Plymouth, the following Lernaeidae were taken:

Haemobaphoides ambiguus T. Scott 1900

Host. *Callionymus maculatus.*
Location. Gills.
Locality. West Rutts—Rame-Eddystone grounds (otter-trawl).
Material. 1 ♀ on 8 hosts examined. First record for English waters.

Saucissona n.gen.

Two species: *S.* [*Lernaea*] *lumpi* T. Scott 1901; *S. sauciatonis* n.sp.

T. Scott 1901 described an animal, of which he had but a single specimen, from the gills of *Cyclopterus lumpus* in the Bay of Nigg, and which he called *Lernaea lumpi*. The generic names *Lernaea* and *Lernaeocera* having been interchanged in accordance with the rules of priority it seems unsuitable to put in the genus *Lernaeocera* an animal whose horns do not branch, and whose trunk and abdomen are neither sigmoid nor bent in a single flexure, but which are in an almost direct line, like a string of sausages, and of which the author stated that "the structure of this species seems in some respects to approach more nearly to that of *Pennella* than is the case with the adult *Lernaea*." Accordingly I have associated it with the new species described below in the new genus *Saucissona* whose diagnosis is as follows:

Head globular; horns three, unbranched; neck, trunk and abdomen without flexures, almost in a direct line, but separated from one another by constrictions; antennules simple in three divisions, not distinctly articulated; antennae chelate; pereiopods as in *Lernaeocera*.

S. sauciatonis n.sp.

Host. *Gobius* (*Pomatoschistus*) *elongatus* Canestrini (*vide* Lebour (1919)).
Location. Gills.
Locality. Eastern end of Rame-Eddystone grounds (Agassiz trawl).
Material. 1 ♀ on 8 hosts examined.

Diagnosis (Fig. 1). Total length 7 mm. Head globular with cruciform markings when viewed anteriorly. *Cephalothorax* bent slightly forward and bearing three horns, one dorsal very much the longest, and two lateral, all

slightly curved and with rounded ends, not straight and sharp-pointed as in *S. lumpi*—the horns curve towards each other to form a grab to enclose the gill arch. The rest of the animal is bright red. *Neck* swollen, three-quarters as long as and broader than trunk, enlarged anteriorly and particularly posteriorly. *Trunk* elongated, slightly constricted in two places, and curved

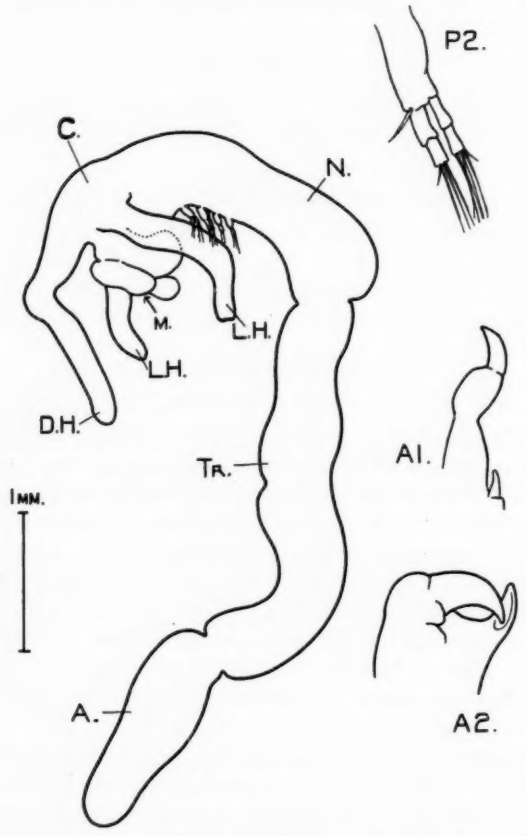

Fig. 1. *Saucissona sauciatonis*, ♀, n.sp. *C*, cephalothorax; *N*, neck; *Tr.* trunk; *A*, abdomen; *D.H.* dorsal horn; *L.H.* lateral horns; *M*, mouth; *P* 2, second pereiopod; *A* 1, antennule; *A* 2, antenna.

anteriorly at the posterior end. Egg-strings wanting. *Abdomen* elongated, almost in line with the posterior end of trunk, rounded posteriorly and almost of the same width throughout. *Antennules* simple, in three divisions without distinct articulations terminating in a blunt claw. *Antennae* chelate, the powerful claw stout and not greatly curved, fitting into projecting socket probably two-articled, but the proximal article cannot be made out without

spoiling the specimen. *Pereiopods*, four pairs close to one another situated immediately behind the cephalothorax, the first two pairs biramous, the second two pairs uniramous, the rami being biarticulate and terminating in three long and one short setae, a seta on the protopodite (probably of all).

Lernaeocera mulli n.sp.

Host. Mullus barbatus.

Location. Gills.

Locality. West Rutts—Rame-Eddystone grounds (Otter trawl).

Material. 2 ♀♀ on 6 hosts examined.

Diagnosis (Fig. 2) (cf. Leigh-Sharpe (1933), Figs. 1 and 2). Horns three, branched, in both specimens probably abnormal, the dorsal one which is con-

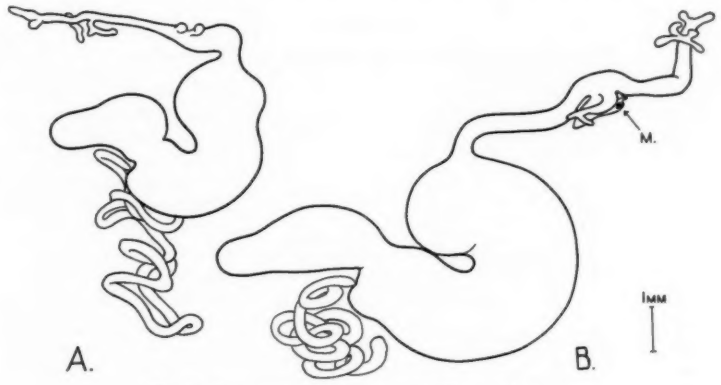

Fig. 2. *Lernaeocera mulli*, ♀, n.sp. *M*, mouth.

siderably the longer, has in each case come up against the bone of the gill arch and exhibits (Fig. 2 A) the common abnormality seen in *L. lusci* and discussed by me (1933, p. 115). The lateral horns are short and at the present stage only fork once (Fig. 2 B). The body however, differs from *L. lusci* (Bassett-Smith), and the animal is from a host not related to the Gadidae to which *Lernaeocera* is, with one exception (*Callionymus lyra*) confined. The cephalothorax is enlarged as in *L. megalocephala* Scott. The specimen A appears to possess no neck, but in the specimen B the neck is elongated without annulations. The anterior part of the trunk is inflated as in *L. phycidis* Leigh-Sharpe 1933, the posterior part is only slightly curved as in *L. lusci*. The abdomen is separated from the trunk by a deep constriction, swollen, much enlarged anteriorly and is bluntly rounded terminally. Egg-strings very long and coiled (not all shown in the figure).

(Type specimens deposited in the British Museum.)

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(*MS. received for publication* 2, VI. 1934—Ed.)

A COMPARISON OF THE PHYSIOLOGY OF THE TWO SPECIES OF BED-BUG WHICH ATTACK MAN

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(With 3 Figures in the Text)

CONTENTS

	PAGE
Introduction	111
Experimental work:	
Analysis of body composition	112
(*a*) Dry matter	112
(*b*) Other constituents	113
Rate of loss of water	113
The thermal death-point	113
The developmental zero	114
Time taken by bed-bugs' eggs to hatch at several temperatures	115
Effects of temperature on the rate of development	116
Effects of humidity on the survival of starved bed-bugs	117
Effects of humidity on fed bed-bugs	118
Discussion	120
Analysis of results	120
Geographical distribution	120
Summary	121
References	122

Introduction

Cimex lectularius is the principal species of bed-bug in temperate countries and *C. rotundatus* is the principal species in the tropics. Anatomically, these two insects are very similar. The general appearance of their bodies, their size and coloration are so much alike that they are not easily distinguished without a lens. The species appear, however, to be quite distinct, and no hybrid has yet been produced. The description given in Patton and Cragg's textbook (Patton and Cragg, 1913) shows that the main distinguishing feature is the shape of the prothorax. In *C. lectularius* it is "semilunar in shape, with two rounded horns which extend close up to the eyes; middle portion of dorsal surface raised, with the sides markedly flattened from a point just external to the level of the eyes." In comparison, the prothorax of *C. rotundatus* is "narrower and shorter, and rounded to the margin." This distinction is easily made out under a low magnification, and is constant for each species. *C. lectularius* is often the larger

insect, but both bugs vary greatly in size. The anatomy and habits of the two species are very similar, and yet they have different geographical distributions. This paper is the account of an investigation to see whether there are any differences in physiology which account for the differences in distribution.

In a previous paper (Mellanby, 1932), I described certain experiments showing how temperature and humidity affect the metabolism of *C. lectularius*. Similar experiments were made with *C. rotundatus*, and the two bugs compared with regard to many of their reactions to climatic factors. Finally, the literature was studied, and specimens in museums, etc., examined, to determine the geographical distribution of the two species. As will be shown, there is some measure of correspondence between the laboratory experiments and the field observations—in fact, the geographical distribution of each species depends, in part, on its physiological reactions.

Experimental work

The *C. lectularius* used in these experiments were derived from a stock which has been bred in this department for over four years. It is of English origin. The *C. rotundatus* were bred from individuals sent by G. H. E. Hopkins from Uganda. As the *C. rotundatus* were fed by the culture tubes being attached to the ear of a lop-eared rabbit, certain observations described in the previous paper (Mellanby, 1932) were repeated using rabbit-fed *C. lectularius*. Previously these insects had been fed on man. The bugs acted in the same way whether fed on rabbit or man.

Analysis of body composition

(*a*) *Dry matter.*

Individuals of both species were weighed separately, dried to constant weight at 105° C. and weighed again. From these figures the proportion of dry matter in their bodies was calculated. (The weighings were made by attaching the insects to pieces of fine copper wire, and hanging them on to a torsion balance. Results were accurate within 1 per cent. A full description of this method is given in the earlier paper.) Fifty-six *C. lectularius* contained 32·42 (P.E. $\pm$ 0·52) per cent. of dry matter, and sixty-three *C. rotundatus* contained 29·50 (P.E. $\pm$ 0·52) per cent. This difference appeared to be a real one, and was found constantly in bugs reared under different conditions. No physiological explanation of it is offered, nor is its importance (if any) understood. It cannot, however, be of great importance, as starvation of either species under special conditions of humidity will cause great changes in the water : solid ratio without apparently damaging the insects. The adult *C. rotundatus* were on an average smaller, their mean weight being 3·90 mg. against 4·82 for the other species. These weights are those of "young" adults, which had only taken one blood meal since their last moult. After a number of feeds the gross weight of the insect rises, but the mean dry matter to water ratio remains fairly constant. The bugs were weighed 2–3 days after feeding—by which time excretion has stopped.

(*b*) *Other body constituents.*

Specimens of both species of bed-bug were analysed by methods used previously for *C. lectularius*. No essential differences were observed, either between the two species, or between individuals of *C. lectularius* fed on man or on rabbit. Small and irregularly occurring amounts of fat were found in both species, and during starvation they both used reserves of protein (blood) contained in their gut. The analyses gave no indication of differences likely to explain the geographical distribution.

Rate of loss of water

Previous work on *C. lectularius* revealed that if they do not excrete, then any loss of weight during starvation is due to water evaporated. Their metabolism is such that the weight of oxygen taken in to produce metabolic water is the same as the weight of carbon passed out in carbon dioxide. *C. rotundatus* behaves in the same way. *C. lectularius* was previously shown to lose weight (*i.e.* water) at different rates, depending mainly on the humidity of the air. The rate of loss was also shown to be more nearly proportional to the saturation deficiency than to any other measure of humidity. *C. rotundatus* behaves in the same way —results obtained with the two species are not significantly different from one another. Table I compares results obtained under identical conditions. Both species were also exposed to many different conditions, and the results could all be compared in the same way as those which are shown in Table I. There is no difference in the rate at which these two species evaporate water into the air.

Table I. *The weights of adults of* Cimex lectularius *and* C. rotundatus, *after exposure to air with either a relative humidity of* 0 *or* 90 *per cent., expressed as a percentage of their original weights. Temperature* 29° C. *in each case. All insects were exposed for* 4 *days.*

Species	0 % R.H.	90 % R.H.
Cimex lectularius	70·6 (P.E. ±0·95)	85·2 (P.E. ±1·20)
C. rotundatus	68·4 (P.E. ±2·30)	86·6 (P.E. ±0·84)

The thermal death-point

Experiments were made to determine the highest temperatures which adults and eggs of the two species would survive, for periods of 1 and of 24 hours. The effects of humidity were also determined. The apparatus used has already been described (Mellanby, 1934). The bugs (or their eggs) were put in small phosphor-bronze cages, which fitted inside glass jars. The humidity in each jar was conditioned by having a layer of the appropriate mixture of sulphuric acid and water at the bottom. The temperature was regulated by submerging the jar in a tank of water which was thermostatically controlled at the required temperature. Adult bugs under a week old, and eggs which had been laid under 24 hours, were used in each case. No adults were killed by exposure for one hour to 42° C. At 43° C. a few died, but the majority survived, while at 44° C.

all were killed. In the 24-hour experiments, all the bugs survived 39° C. and all died at 40° C. These results are true for each species, and the thermal death-point was the same at every humidity. Eggs were exposed in the same way, after which they were incubated at 23° C. to see how many would hatch. Controls were also kept, over 95 per cent. of which hatched. The survival temperature of the eggs of both species was one degree higher than that of the adults, in exposures of either 1 hour or 24 hours. Humidity did not affect the death-point of the eggs.

These results indicate that the adults and eggs were both killed by the effects of heat alone. Although those exposed to dry air lost more water than those exposed to moist (see p. 113 above), both the adults and eggs lose water slowly, and after 24 hours' exposure to dry air at a temperature which is sublethal, they still contain sufficient water to maintain life. In experiments lasting for longer than 24 hours, effects of humidity on the thermal death-point would no doubt be apparent.

Bed-bugs are killed by the heat at temperatures which often occur in tropical regions. Sometimes this must cause their death, but they will frequently escape because they live in damp cracks in walls, etc., where evaporation reduces the temperature, and produces a special micro-climate (see Mellanby, 1933). As the death-points of both species are identical, as far as survival of high temperatures is concerned, *C. rotundatus* is no better adapted to life in the tropics than is *C. lectularius*.

The developmental zero

Bed-bugs do not hibernate in the true sense of the word, but they remain dormant at low temperatures. When the temperature rises, activity starts once more. The minimum temperature at which bugs show signs of activity is approximately the same as the developmental zero for the eggs. But whereas the developmental zero is sharply defined for each species, some activity may be found among bugs which are disturbed at lower temperatures, and for several degrees above the zero temperature both adults and nymphs may be extremely sluggish.

As Jones (1930) has already shown, the developmental zero for *C. lectularius* is approximately 13° C. This was confirmed for the stock used in these experiments. Adult *Cimex* were kept at 23° C., fed twice a week, and their eggs removed daily for use in these experiments. At 10° C. no development took place, but at 14° C., out of 150 eggs, 66 per cent. hatched on an average in 47 days (P.E. $\pm$0·18). The eggs which did not hatch were found to contain partly developed embryos. At 15·9° C., 91 per cent. hatched, in 29·9 days (P.E. $\pm$0·15).

C. rotundatus has a higher developmental zero. At 14° C., no eggs hatched, and after leaving one batch at 14° C. for 35 days, they were transferred to 30° C. The eggs were dead. They did not develop or hatch, though a batch of *C. lectularius* eggs treated in the same way hatched within 2 days. Only

19 per cent. of a large batch of *C. rotundatus* eggs kept at 15·9° C. hatched, and they took 33·6 days (P.E. ± 0·7).

It appears that the eggs of the species of bed-bug associated with temperate regions can develop and hatch at lower temperatures than those of the tropical species. The developmental zero of *C. lectularius* is approximately 13° C., while that of *C. rotundatus* is 15° C.

Time taken by bed-bugs' eggs to hatch at several temperatures

Eggs of bed-bugs were obtained as described in the last section of this paper. They were subjected to several different temperatures. It was not necessary to control the humidity in all cases, for it was found that below 30° C. the eggs hatched in the same time, and in as great numbers, whatever the humidity. Even in quite dry air at 30° C., when the saturation deficiency was 31·7 mm., over 95 per cent. of the eggs of both species hatched. The eggs must lose water very slowly.

At 23° C., *C. lectularius* eggs were found to hatch in 7·92 days (P.E. ± 0·30), while those of *C. rotundatus* took 9·01 days (P.E. ± 0·27). In both cases over 200 eggs were used.

At 30° C., the eggs of both species hatched in 4 days. Occasionally one or two eggs out of a batch of twenty-five which did not hatch on the fourth day hatched later, but this was exceptional. At 30° C. nearly 100 per cent. of the eggs hatched. The eggs of the two species behaved in the same way.

At temperatures above 30° C., the eggs never hatched in less than 4 days. At 34° C., approximately 80 per cent. of the eggs of both species hatched (in 4 days), and partly developed embryos were seen in the other eggs.

Exposure for 24 hours to 41° C. killed eggs of both species. At temperatures between 35 and 40° C., continued exposure proved fatal in most cases, although partial development of embryos frequently took place. A great many experiments were made at a temperature of 37° C. If eggs of either species were kept at this temperature all the time, embryos developed partially, but none hatched. It was found that if the eggs were incubated for 2 days at 30° C. and then transferred to 37° C., nearly all of them hatched, provided that the relative humidity was above 60 per cent. Although the eggs could withstand considerable desiccation, there are evidently limits to the amount of water they can lose unharmed. Eggs also hatched when they were kept for 2 days at 37° C. (in fairly moist air) and then transferred to 30° C. The above observations apply to both species. The eggs of *C. rotundatus* appeared to be slightly better than those of *C. lectularius* at withstanding high temperatures; 60 per cent. hatched after 3 days at 37° C. and 1 day at 30° C. (it did not matter whether the exposure to 30° C. occurred at the beginning or end of the experiment), while under 5 per cent. of *C. lectularius* eggs hatched in a similar experiment. High temperatures appear in some way to be unfavourable, and the unfavourable effect depends on the length of the exposure. It is not the hatching mechanism

alone which is affected—it looks as though excessive heat produced some harmful substance.

The results described in this and the previous section are shown graphically in Fig. 1. The dotted line represents the time taken by eggs of *C. rotundatus* to hatch at different temperatures. The plain line represents the time taken by *C. lectularius*. In each case, "*Z*" represents the developmental zero. *C. lectularius*

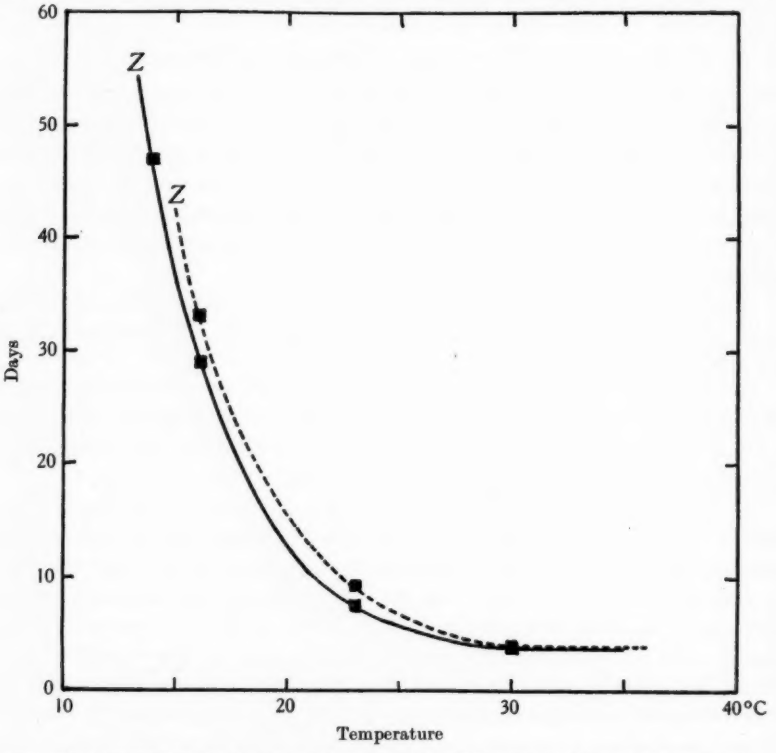

Fig. 1. The time required by the eggs of the two species of bed-bug to hatch at different temperatures. The dotted line is used for *C. rotundatus* and the plain line for *C. lectularius*. *Z* represents the developmental zero in each case.

is able to start development at a lower temperature than can *C. rotundatus*, and below 30° C. it develops more rapidly. Above 30° C. the two species behave in the same way, except that *C. rotundatus* may be slightly more resistant to high temperatures.

Effects of temperature on the rate of development

Eggs of both species of bed-bug were kept at 20, 23 and 30° C., and in most cases the relative humidity was 60 per cent. (For effects of humidity, see p. 118 below.) When the eggs hatched, the nymphs were given the opportunity to feed on alternate days—which meant that all which were ready to feed were able

to do so as often as possible. The bugs were examined regularly, and the times taken in developing from egg to adult were found.

At 20° C., *C. lectularius* took between 63 and 67 days (counting from the day on which the egg was laid) to reach the adult stage; thirty-five *C. rotundatus* eggs had only produced one adult (and thirty-four nymphs of various stages) by the 68th day, when the experiment had to be stopped due to an accident to the thermostat of the incubator. Evidently, at 20° C., *C. lectularius* is able to develop quicker than *C. rotundatus*.

At 23° C., the mean period for development from egg to adult for *C. lectularius* was 45 days. The shortest time observed was 40 days, and the longest 52 days. For *C. rotundatus*, the average time was 53 days (limits 46–66 days).

At 30° C., the mean period for *C. lectularius* was 25 days, and for *C. rotundatus* 27 days.

Table II. *Length of survival in days of newly hatched nymphs, exposed to different temperatures and humidities. Some of the nymphs were unfed, others gorged with blood. The probable error follows each result.*

Temperature °C.	Fed or unfed	R.H. % 0	30	60	90
		Cimex lectularius			
30	Unfed	5·68 (±0·10)	8·8 (±0·22)	11·6 (±0·52)	26·3 (±2·20)
	Fed	9·96 (±0·25)	11·28 (±0·39)	14·47 (±0·64)	28·73 (±1·66)
36	Unfed	3·89 (±0·12)	4·11 (±0·27)	6·81 (±0·32)	15·74 (±1·18)
	Fed	6·67 (±0·42)	5·53 (±0·43)	12·6 (±0·62)	15·53 (±0·47)
		C. rotundatus			
30	Unfed	6·0 (±0·20)	6·85 (±0·50)	13·25 (±0·41)	15·95 (±0·29)
	Fed	9·26 (±0·71)	13·68 (±0·70)	20·64 (±0·34)	28·96 (±2·60)
36	Unfed	2·65 (±0·13)	3·2 (±0·22)	3·81 (±0·16)	4·50 (±0·16)
	Fed	6·94 (±0·44)	9·94 (±0·49)	9·31 (±0·54)	9·81 (±0·60)

It was thought that the difference might be due to *C. lectularius* taking rabbits' blood more readily than the other species, but when *C. rotundatus* were fed on man, their behaviour with regard to climatic factors was unchanged. The experiments show that as a rule it is possible for *C. lectularius* to develop more rapidly than *C. rotundatus*, if the conditions of temperature are the same. At lower temperatures, it may be said that *C. lectularius* is proportionately quicker—at higher temperatures *C. rotundatus* almost "catches up." *C. lectularius* is the better adapted to temperate conditions.

Effects of humidity on the survival of starved bed-bugs

Bed-bugs of both species are well known to be able to survive long periods of starvation. The experiments of Jones (1930) on *C. lectularius* show how great the effect of humidity is on the survival of newly emerged nymphs. Further experiments were made in which nymphs of both species were exposed to 30 and 36° C., and to various definite humidities at both temperatures. The periods which the species survived, both with and without having first had one blood meal, were discovered. The results are shown in Table II.

Consider first the results obtained using *C. lectularius*. At 30° C. the unfed nymphs survived different periods, depending on the humidity. With air of 90 per cent. relative humidity, they survived 26·3 days. In this case, death was due to the exhaustion of some reserve (in fact, starvation) and not due to desiccation. At the lower humidities, however, desiccation was the principal cause of death, and careful analysis of results reveals that the water was probably lost at a rate proportional to the saturation deficiency. This conclusion could only be drawn by considering results in which the *sole* factor causing death was obviously desiccation. Now, when similar nymphs were allowed to gorge with blood, the survival times in dry air were increased. Obviously then, the blood must have acted as a supply of water. In moist air, however, the survival time was not significantly altered—so it appears that *C. lectularius* nymphs do not survive starvation (in distinction to desiccation) any better after feeding. The blood meal no doubt has two effects. As well as supplying food material, it stimulates metabolism (the bug moults, etc.), and so the food material is rapidly utilised. When *C. lectularius* nymphs were exposed to 36° C., results were obtained which may be interpreted in the same way as those obtained at 30° C.

Turning now to the figures obtained using *C. rotundatus*, the main difference that is seen is that in the experiments in moist air, both at 30 and 36° C., the nymphs which were allowed to feed once survived much longer than those which were unfed; in fact, the blood, as well as supplying water, also supplied food materials which themselves prolonged life.

The results obtained with the two species may now be compared. The first conclusion to be drawn appears to be that unfed nymphs of *C. lectularius* can withstand starvation much better than nymphs of *C. rotundatus*, and they can also withstand more extreme desiccation. After taking one meal of blood, the powers of survival of the two species are approximately the same, though even then *C. lectularius* is slightly more resistant.

These results indicate little adaptation to life in temperate or tropical regions on the part of either species. They do indicate, however, that *C. lectularius* is the more resistant under a variety of conditions.

Effects of humidity on fed bed-bugs

It has been shown that the humidity of the air has important effects on bed-bugs which are not allowed to feed. How does it affect individuals which have regular access to food?

Both species were bred in tubes at 23 and 30° C., and at 0, 30, 60 and 90 per cent. relative humidity in each case. The bugs were given the opportunity of feeding on alternate days. The humidity did not appear to have any important effects under these circumstances. The time the bugs took to develop was the same at one temperature and each humidity, the adults were of the same size and had similar body compositions.

Adult bugs were also kept at 23° C. in air with a relative humidity of either 0 or 90 per cent. The effects of the humidity were found on the size of the bugs, and on their body composition. The bugs were given access to the rabbit once in 5 days, which was about as often as they would take blood when kept at 23° C. Their weights were measured frequently, and many individuals were analysed. In some cases, the amounts of blood taken at meals were measured, together with the weight of excretory products. Results obtained are shown diagrammatically in Fig. 2. The two rectangles marked *A* and *B* represent individuals which were exposed to 90 and 0 per cent. relative humidity respectively. The lower (shaded) parts of the rectangles represents the amount of solid matter present in the bugs at the start of the experiment, and the upper (unshaded) part represents the amount of water present. After 5 days' starva-

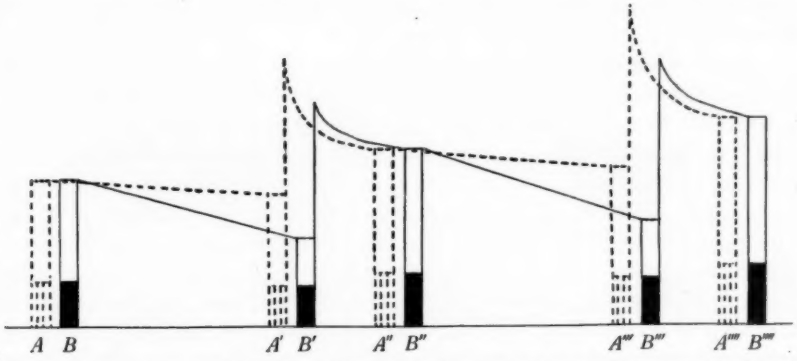

Fig. 2. Diagram to show the effects of different humidities on bed-bugs which received sufficient food. Each rectangle shows the body composition of an individual; the black part represents solid matter, and the white water. All experiments were done at 23° C. The dotted figures were obtained in air with a relative humidity of 90 per cent., and the plain ones in dry air (R.H. 0 per cent.). For further description see text.

tion, the body composition of *A* and *B* altered, and they are represented by the rectangles *A′* and *B′*. They both utilised small and identical quantities of solid matter, but while the bug kept in moist air (*A*) maintained its water: solid ratio constant, *B′* now contains a much lower proportion of water than before desiccation. The vertical lines leading from *A′* to *B′* represent the amounts of blood taken by the two bugs—each takes the same amount. After feeding the rapid excretion that always occurs is depicted. *A*, kept in moist air, produced nearly twice as much watery excretion as *B*. The result is shown as *A″* and *B″*. Both bugs are now the same size, and contain the same proportion of water and dry matter as they did at the start. Thus, by controlling excretion of superfluous water, bed-bugs which are fed frequently can maintain their water balance in spite of the humidity of the air. The results of a further period of desiccation followed by another feed are also shown (*B‴* and *B⁗*). With each successive feed the adult bug becomes heavier. Fig. 2 is simplified by the omission of egg

laying, but frequently this does not start until after two feeds have been taken. Fig. 2 is based entirely on experiments made with adult bugs, but it also explains why cultures may be bred equally successfully at all humidities, provided they are fed often enough. The facts depicted in Fig. 2 are true for both species of bed-bug.

Discussion

Analysis of the results

The results which have been described show many physiological similarities between *C. lectularius* and *C. rotundatus*. There is no fundamental difference in body composition, nor in regard to water balance. The thermal death-point is the same in each. Both species are able to develop over a wide range of humidity, which does not affect the culture provided there is sufficient opportunity of feeding.

On the other hand, there are several differences between the two species. *C. lectularius* is evidently able to develop at lower temperatures, and to develop at a greater rate than *C. rotundatus* at all temperatures below 30° C. This means that if the two species were competing in any area, *C. lectularius* would be at a considerable advantage. Even in the tropics, the temperature frequently falls considerably below 30° C. (86° F.), and as soon as that happens any *C. lectularius* should breed, etc., more rapidly than *C. rotundatus*. In these experiments, the tropical species only showed one adaptation to high temperatures—its eggs could survive longer exposures to 37° C. than could those of the temperate species. But on the whole, while *C. lectularius* is the better adapted to living in temperate regions, if introduced to the tropics it should do at least equally as well as the indigenous species.

Geographical distribution

It did not prove easy to determine accurately the geographical distribution of the species of bed-bug. Most of the data given in the literature[1] is of a vague nature, as it has not been collected for this purpose. I have made a fairly complete study of the literature, and examined specimens of bugs in museums, etc., collected from different parts of the world. The results obtained are summarised in Fig. 3. This map is certainly far from complete, but certain facts appear. *C. rotundatus* is restricted to tropical and subtropical regions. It appears to be an Old World insect which has been spread to the Pacific Islands and South America fairly recently. If it is introduced in the tropics, it appears well able to establish itself. *C. lectularius* also appears to be an Old World species, coming originally from Asia. It has been spread to all other continents. It is most commonly found in temperate regions, but it is also found in ports in the tropics, where it is establishing itself and spreading (Horvath, 1913).

[1] It is not practicable to give the reference to all the papers consulted. Those interested should see Horvath (1913) and also the summaries published in the *Review of Applied Entomology* (B).

The distribution of *Cimex* seems to depend to a great extent on how it is dispersed. America, Australia and South Africa have all been infested with *C. lectularius*. These have been carried there by man. Fewer *C. lectularius* have been carried to the tropics, because fewer Europeans likely to carry bugs have emigrated there. On the other hand, when labourers from tropical and sub-tropical countries emigrate, they emigrate usually to other tropical regions, and take their bed-bugs with them. In this way Southern Chinese introduced *C. rotundatus* into Samoa (Furguson, 1926). But the results published in this paper show that it is unlikely, even if successfully introduced, that *C. rotundatus* could become an important pest in temperate countries. On the other hand, where *C. lectularius* has been introduced into the tropics, it is likely to spread considerably.

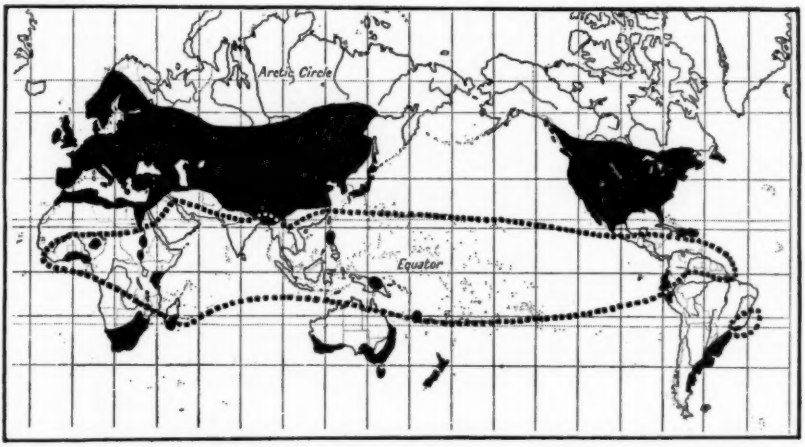

Fig. 3. Map to show the geographical distribution of the species of bed-bug. The black areas show where *C. lectularius* has been found. *C. rotundatus* is only found within the areas enclosed by the dotted lines.

SUMMARY

The two species of bed-bug, *Cimex lectularius* and *C. rotundatus*, are found in temperate and tropical regions respectively. They are very similar anatomically, and have the same habits.

Physiologically, the two species have many resemblances. Their water balance is similar; they are very little affected by humidity if there is plenty of food available. The thermal death-points are identical.

At temperatures below 30° C., *C. lectularius* is able to breed more rapidly and more successfully than *C. rotundatus*. Between 30 and 35° C. both species behave alike, and above 35° C. *C. rotundatus* may be the more efficient species. Under most conditions, *C. lectularius* is the more resistant to starvation and desiccation.

Results indicate that although *C. lectularius* is more fitted for life in tem-

perate countries, if introduced into the tropics it should be able to carry on at least as well as *C. rotundatus*.

Studies of geographical distribution indicate that *C. rotundatus* has spread all over the tropics, but has never established itself outside them. *C. lectularius* has spread all over the temperate world, and into the ports in some tropical countries. Experimental work suggests that it may become widespread in those parts of the tropics into which it is introduced.

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(*MS. received for publication* 21. VII. 1934.—Ed.)

IXODES RICINUS IN RELATION TO ITS PHYSICAL ENVIRONMENT

II. THE FACTORS GOVERNING SURVIVAL AND ACTIVITY

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(With 8 Figures in the Text)

CONTENTS

		PAGE
I.	Resistance to unfavourable conditions	124
	(*a*) General	124
	(*b*) Cold resistance	124
	(*c*) Thermal death-point	125
II.	Climatic limits for survival	125
III.	Influence of the humidity factor on duration of survival within the critical temperature limits	127
	(*a*) Comparative survival of nymphs and larvae	127
	(*b*) Intereffects of temperature and humidity	129
	(*c*) Relation of saturation deficiency to loss of water	131
	(*d*) Saturation deficiency, loss of water and survival	135
IV.	Influence of temperature on activity	137
	(*a*) Rate of activity	137
	(*b*) Preferred temperature	138
V.	The phototropic response at different temperatures and humidities	139
VI.	The influence of temperature on geotropic response	141
VII.	Discussion	142
	References	143

In a previous paper (MacLeod, 1934) the different physical factors in the environmental complex of the sheep tick were discussed in their relation to the development of the gorged tick, and an experimental analysis was made of the effects produced, under laboratory conditions, by their action and interaction. The present paper deals with the active unfed ticks. These present rather a different problem from the gorged ticks, for, whereas in their case the climatic optimum resolved itself into that combination of conditions under which development proceeded favourably and with a low attendant mortality, the unfed tick, on the other hand, is faced with two desiderata, survival and host-parasitisation, the physical optima for which may not be coincident. It is necessary, therefore, to consider not only the climatic optimum for survival and activity, but also the factor or factors which influence parasitisation, and those conditions under which host-parasitisation is facilitated.

The methods used for controlling temperature and humidity were similar to those already described. Special methods for different purposes will be given in the text under the description of the experiments concerned.

I. Resistance to unfavourable conditions

(*a*) *General*

The ability of this tick to survive unfavourable conditions is remarkable; in the absence of a suitable host, the adult has been found to survive without a meal for thirty-one months; nymphs and larvae were able to engorge after fasting thirteen and fifteen months respectively (MacLeod, 1932). Gibson-Carmichael (1884) stated that he had kept females under water from 2 to 13 days, and that the ticks did not show any weakness on being removed. Olenev (1927) records that larvae which hatched under water and remained submerged, survived for 1½ months, and Totze (1933) says that larvae were unaffected by submersion for 80 days. The writer kept females submerged for 21 days at a temperature of about 15° C. On being removed, they walked away apparently unaffected.

(*b*) *Cold resistance*

Different unfed stages were placed in small vessels containing dampened sand, and the vessels placed in a freezing chamber. The temperature of the chamber was lowered a few degrees each successive day. Similarly, after exposure for the required period, the ticks were gradually brought up through daily increases of temperature to that of the laboratory, and there examined for death or survival. Table I gives the results of exposure of the three stages to temperatures varying from 0° to −15° C. It will be seen that the tick in all its stages can survive exposure for 4 days to a temperature of −8° C. It is killed by 1 day's exposure to a temperature of −15° C.

Table I. *Resistance to low temperatures.*

Commencing temperature ° C.	Reduced during (days)	Final temperature in ° C.	Exposed for (days)	Raised during (days)	Result
		Larvae			
16	1	0 to 3	45	1	Survived
	3	−5 to −4	3	—	,,
	5	−8 to −4	10	2	,,
	5	−7 to −5	6	2	Killed
	3	−8	4	—	Survived
	3	−12	3	2	,,
	7	−14 to −12	1	6	,,
	8	−12	2	4	Some survived
	7	−15	1	6	Killed
		Nymphs			
16	3	−5 to −4	3	—	Survived
	3	−8	4	—	,,
	3	−14 to −12	2	4	Killed
	8	−14 to −12	1	6	,,
	3	−12	3	2	Survived
	7	−15	1	6	Killed
		Females			
16	3	−5 to −4	3	—	Survived
	3	−8	4	—	,,
	3	−14 to −12	2	4	Killed
	8	−14 to −12	1	6	,,
	3	−12	3	2	Survived
	7	−15	1	6	Killed

Table II gives the comparative resistance to low temperatures of recently hatched and older larvae. It will be seen that larvae which have been hatched for some time are able to survive lower temperatures and for longer periods than larvae recently hatched.

(c) *Thermal death-point*

With increasingly high temperatures, the influence of humidity in prolonging the survival period of the exposed ticks steadily diminishes. Thus, nymphs at 35° C. survived 3–4 days at 70 per cent. saturation, 11–14 days at 100 per cent., and survived indefinitely at 90 per cent. At 37·5° C. they were

Table II. *Resistance to low temperatures of older versus newly hatched larvae.*

Stage	Temperature ° C.	Duration of exposure (days)	Result
1–7 days	0	7	Survived
	−9 to −4	3	Killed
	−9 to −4	2	,,
7 weeks old	−11 to −9	5	Some survived
	−11 to −9	6	Killed

killed at all humidities by 15 days' exposure, surviving less than 1 day at 70 per cent., and 6–9 days at 90 per cent. At 45° C. they were killed by exposure for 1 hour at 70, 90 and 100 per cent. Larvae exposed at 35° C. survived less than 1 day at 70 per cent., and were killed in 11–14 days at 90 per cent. and 100 per cent.; at 37·5° C. they were killed by exposure for 2 days at 90 per cent. and 6–9 days at 100 per cent. They were killed by 24 hours' exposure in saturated air at 40° C. and 22–54 min. exposure at 45° C.

It would appear therefore that at 35° C. larvae are less resistant than nymphs at humidities below saturation, but equally resistant in saturated air. At 37·5° C. the same holds good, but the humidity range within which there is a differential resistance is much more limited. At 45° C. both stages were killed by 1 hour's exposure, regardless of humidity. Since the nymph is tracheate, and the larva non-tracheate, their differential resistance at humidities below saturation is probably related to the difference in their mechanisms for water exchange with the surrounding air. The temperature at which the difference in their survival periods is no longer apparent will therefore correspond with the thermal death-point, at which humidity ceases to exercise any effect on survival. This point would appear to lie, for the tick, in the neighbourhood of 40° C.

II. CLIMATIC LIMITS FOR SURVIVAL

The duration of survival of nymphs and larvae was tested at temperatures of 2, 10, 15, 20, 22·5, 25, 27·5, 30, 32·5, 35 and 37·5° C., and at humidities of 0, 10, 30, 50, 60, 70, 75, 80, 85, 90, 95 and 100 per cent., at each temperature. The combinations of temperature and humidity were obtained by the methods previously described, with the addition that 0 per cent. humidity was obtained by the use of anhydrous P_2O_5. The ticks were confined in test-tubes, the mouths

of which were covered with fine-mesh muslin, and these placed in the different climate systems, which were then sealed. To avoid fluctuations of temperature and humidity, they were examined only at periods of 1, 2, 5, 10 and 15 days. Results were noted either as "survived" or 100 per cent. death. About 100 larvae were used in each system; all of them originated from the same egg cluster. Unfortunately, the number of available nymphs of the same age was limited, so only four were used at each climate combination. This was compensated for, however, by the large number of records obtained, as a result of

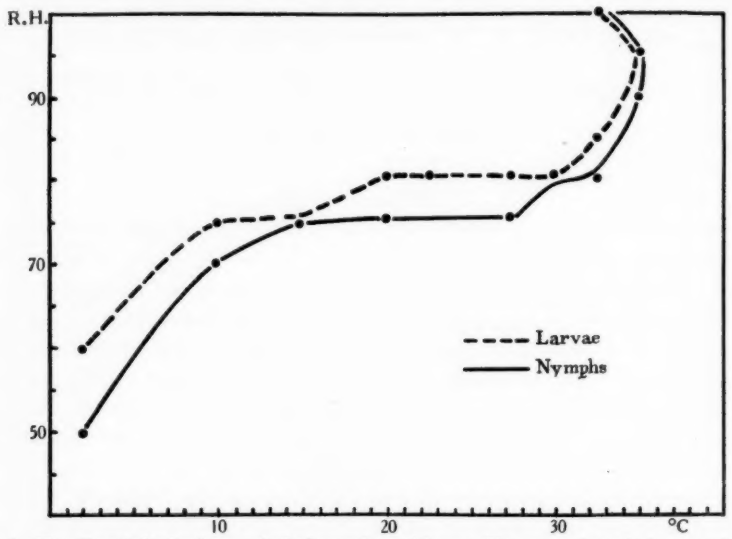

Fig. 1. The climatic limits for survival of nymphs and larvae. The curves join the lowest relative humidity for each temperature at which any ticks survived for 15 days.

the extreme closeness of the intervals in both the temperature and humidity scales.

The experiment was discontinued after 15 days. Fig. 1 shows the limits at which some ticks were still alive after this time. The curves are obtained by joining the points representing the lowest humidity for each temperature at which survival occurred. These curves may be taken as indicating the approximate limits of temperature and humidity at which the tick will survive. Here again, as in the case of the limits for development of the gorged tick, the most striking feature is the narrow range of humidity within which survival is possible. Even at a temperature of 10° C., the lower humidity limit for survival of both stages lies between 70 and 75 per cent., while at temperatures of 15–30° C. it lies between 75 and 80 per cent. It is to be remembered also that this limit represents that below which 100 per cent. death occurred in 15 days, so the limit for 100 per cent. or even 50 per cent. survival over a longer period will be even higher.

These findings are in agreement with those of Olenev (1927), who, working with temperatures varying between 10 and 20° C., found that, at 70 per cent. R.H., 50 per cent. of the larvae were dead by the sixth day, 75 per cent. by the ninth and 100 per cent. on the tenth day.

The nymphs appear to be slightly more tolerant of dry conditions than the larvae, while, at the upper temperature limit of 35° C., both stages die in saturated air while surviving in air 95 per cent. saturated.

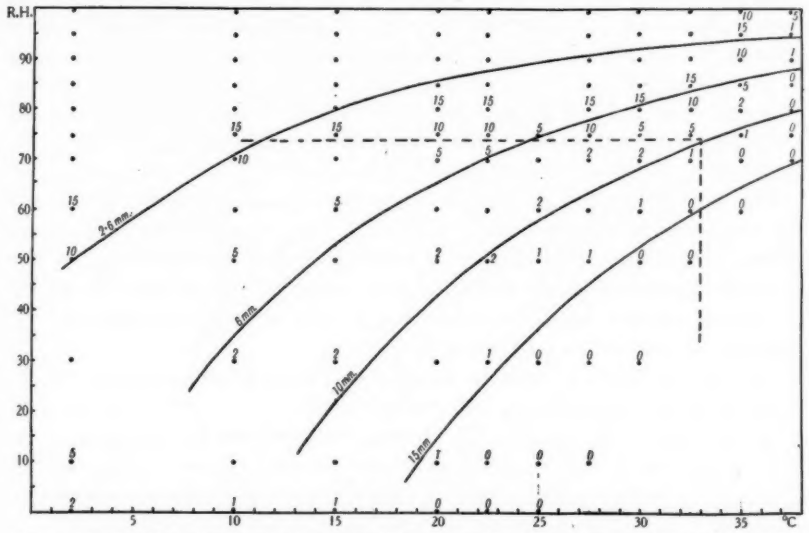

Fig. 2. The duration of survival of larvae at different combinations of temperature and humidity. The points indicate the combinations at which survival was tested. Only the lowest relative humidity at each temperature for survival for periods of 15, 10, 5, 2 and 1 day respectively have been indicated by the appropriate figure.

III. INFLUENCE OF THE HUMIDITY FACTOR ON DURATION OF SURVIVAL WITHIN THE CRITICAL TEMPERATURE LIMITS

(*a*) *Comparative survival of nymphs and larvae*

If the survival periods of the tick are plotted on a chart, of which the ordinates are temperature and relative humidity, the intereffects of these factors on the duration of survival becomes clear. Figs. 2 and 3 represent the survival of larvae and nymphs respectively at different combinations of temperature and humidity. Where the ticks were killed in 1 day, the figure 0 is noted. If they survived 1 day, but were killed in 2 days, the figure 1 is entered, and so on. To simplify the figure, only the lowest humidity point for survival at each temperature is noted. The dots indicate the combinations at which survival was tested. Across the diagrams lines have been drawn corre-

sponding to certain fixed saturation deficiency values. Fig. 2 illustrates several points of interest.

(1) At unfavourable humidities, *i.e.* humidities of 70 per cent. or lower, larvae at any fixed relative humidity survive longer at low temperatures than at high.

(2) Within certain limits of temperature and humidity—marked on the diagram by interrupted lines—the duration of survival appears to be related to saturation deficiency. Thus, at temperatures of below 30° C., the lower humidity limit for 1 day's survival corresponds with a saturation deficiency of 15 mm. Hg, and the limit for 2 days' survival with 10 mm. There is a lesser degree of correlation between the lower limit for 5 days' survival over a range of temperature and any single saturation deficiency value, while at conditions less unfavourable than this, where the tick survives for 10 or 15 days, the limiting humidity cannot be related to this measure.

(3) As the humidity conditions become less adverse, the limit for survival for the appropriate period tends to diverge from saturation deficiency constants, and to approximate more to those of relative humidity until, under favourable conditions, with survival for 15 days, the limit corresponds closely to a fixed relative humidity value over a wide range of temperature, *e.g.* between 10° and 30° C. the limit is 75–80 R.H.

(4) The correlation between saturation deficiency and survival breaks down at high temperatures. At temperatures over 32·5° C., the survival periods are relatively shorter than at lower temperatures for particular limits both of saturation deficiency and of relative humidity, until, as the thermal death point is approached, moisture ceases to have any effect in prolonging survival.

A study of Fig. 3 shows that, in the case of the nymph, survival under highly unfavourable humidity conditions is not related to saturation deficiency, at least at temperatures of over 25° C. Thus, at 32·5° C., the lower limit of saturation deficiency which the nymph can tolerate for 2 days is 15 mm. deficiency, while at a temperature of 27·5° C. the nymph survives for this period at a deficiency of 25 mm. At intermediate conditions, there appears to be some correlation. Thus, the lower limiting humidity for 5 days' survival is fairly closely represented by a saturation deficiency of 10 mm. There is a much lesser degree of correlation, and over a lesser temperature range, between saturation deficiency and the limiting conditions for 10 days' survival—the interrupted line indicates the approximate humidity limit—while there is no agreement between the limits for survival for 15 days and any saturation deficiency.

It is evident that the nymph is more tolerant of aridity than the larva; that is, it would appear to possess some mechanism, absent in the larva, by which it can reduce the rate of loss of water under unfavourable moisture conditions. Since the larva is non-tracheate, loss of water in its case must be from the general body surface. In the tracheate nymph, on the other hand,

loss of water is probably mainly from the tracheal tree. Buxton (1932) has pointed out that tracheate species of arthropods appear to be more resistant to drying than non-tracheate species. It is possible, therefore, that the nymph under arid conditions conserves moisture by closing the spiracles.

It is of interest to compare the humidity limits for survival of the two stages. In Fig. 4 the limits for 1 day's survival are compared; the comparison of the limits for 2 days' survival presents a similar picture. It will be seen that the greater toleration of the nymph becomes less marked at extremes both of aridity and temperature. The convergence of the limits at extreme conditions of aridity is of interest, and suggests a collapse of the water-conserving

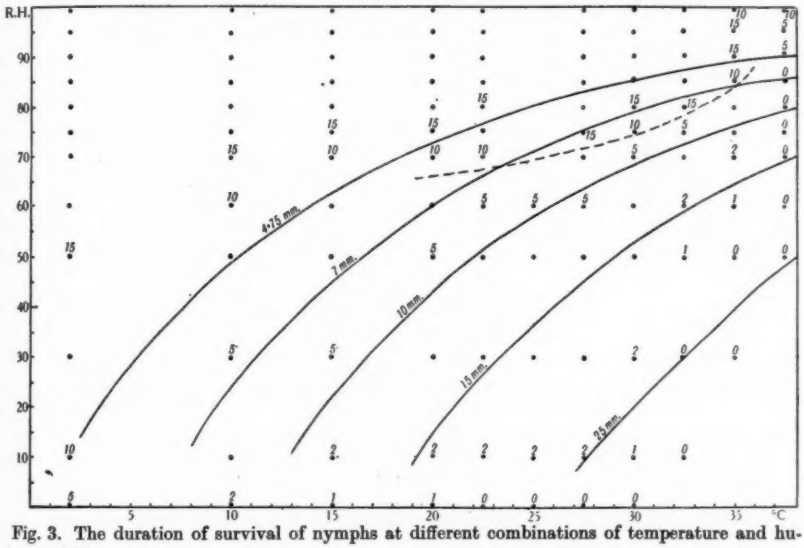

Fig. 3. The duration of survival of nymphs at different combinations of temperature and humidity.

mechanism, so that the nymph loses moisture almost as rapidly as the larva. At the temperature extreme, the difference between the toleration limits of the two stages decreases because the distinctive ability of the nymph to conserve water becomes of decreasing importance, aridity playing a decreasing part in producing death as the thermal death-point is approached.

(*b*) *Intereffects of temperature and humidity*

It has been shown that with increasing temperature the partial effect of heat in causing death increases. At higher temperatures, therefore, the duration of survival will not maintain the same relation to rate of water loss as at lower temperatures. If it be assumed that this harmful effect of heat requires, at a fixed temperature, a fixed time to operate, then even at one temperature, provided this is above the optimum, duration of survival will not be pro-

portional to rate of loss of water. Thus, the slower the rate of water loss the greater proportion of the lethal effect is attributable to heat, death occurring in such case before the maximum amount of water is lost which the organism can tolerate. Therefore, even at one temperature, if this be above the optimum, duration of survival will not be exactly proportional to saturation deficiency, even although the rate of water loss be so. This hypothesis is supported by the following experiment.

Six groups of twenty unfed nymphs were exposed at a temperature of 34° C. to humidities of 90, 70, 50, 30, 10 and 0 per cent. relative humidity. They were examined every few hours, and the number of deaths recorded.

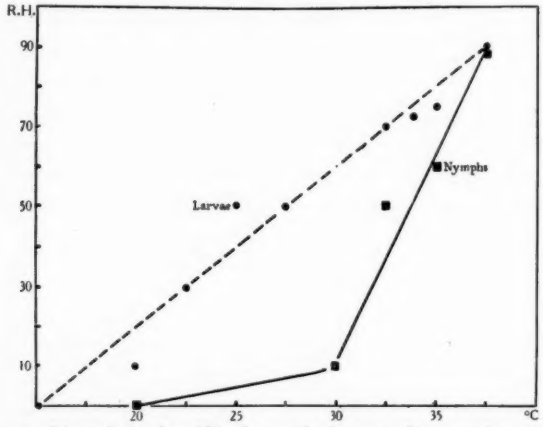

Fig. 4. The lower limiting relative humidity for one day's survival in nymphs and larvae. The one day limit for nymphs at 22·5, 25 and 27·5° C. will lie between 10 and 0 R.H., since they survived 2 days at 10 R.H. and less than one day at 0 R.H.

Fig. 5 shows graphically the duration of survival at each humidity, percentage mortality being plotted against time. From the figure, the time point for 50 per cent. death at each humidity can be obtained.

If the time in hours required for 50 per cent. death is multiplied by the saturation deficiency (S.D.) corresponding to each humidity, the following series of values is obtained:

R.H.	Hours	×	S.D.	=	*P*
70	32	×	11·97	=	383
50	27	×	19·95	=	539
30	20	×	27·93	=	559
10	18	×	35·91	=	646
0	14	×	39·90	=	559

Now, if survival were inversely proportional to saturation deficiency, the product of the two (*P*) should be a constant. In the above experiment *P* is an increasing value with decreasing humidities down to 10 per cent. That is, the tick survives relatively longer at low humidities than at high. This would indicate that with decreasing humidity death becomes due more and more to

loss of water, the heat-lethal effect having increasingly less time to operate, and, consequently, being responsible for an everlessening share in producing death. Therefore, as the cause of death becomes more and more due to loss of water, an increasing proportion of the water content in the body requires to be lost, until the maximum amount is reached which the tick could lose under any conditions, death being then due solely to water loss.

An interesting point is the unexpectedly low P value for 0 per cent. humidity. Here, again, there would appear to be a collapse of the water-conserving mechanism, so that water is lost at a relatively greater rate than at other humidities.

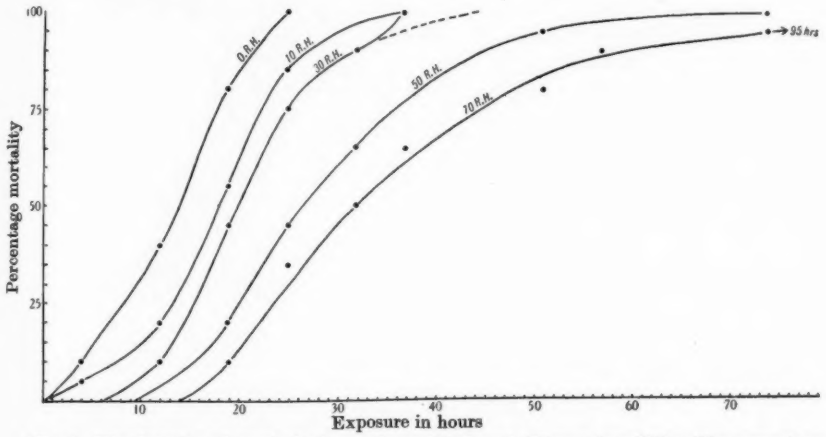

Fig. 5. The duration of survival of nymphs at 34° C. and different humidities. The percentage death is plotted against time in hours.

(c) *Relation of saturation deficiency to loss of water*

It has been shown that survival of the tick can be measured by saturation deficiency only under limited conditions both of temperature and humidity. The deviation at higher temperatures is due to the interference of the heat-lethal factor; there remains to consider the case of deviation at temperatures where this effect might be expected to be inoperative, and death be attributable entirely to loss of water. The question of water loss in insects has received considerable attention in recent years, and it was felt desirable to investigate the question in relation to the tick in some detail, more especially as the tick is peculiarly suited to this type of study. It can live in the unfed condition at room temperature for upwards of a year. Destruction of body tissues by metabolic processes must, therefore, be very slow: over a period of days the loss due to this cause must be almost inappreciable, and so experimental variations in body weight over such periods can justifiably be regarded as simple effects of external environmental causes. Further, under unfavourable moisture conditions, death can be shown to be due to loss of water from the

tissues. In *Tenebrio* and in *Rhodnius*, Buxton (1930 and 1932 *a*) has shown that loss of water from the tissues is compensated by increased production of metabolic water, so that the ratio of dry weight to total weight remains constant. Death will, therefore, be due more to exhaustion of tissues than to simple water loss, and, therefore, because of gaseous interchange, the loss of weight of the organism cannot strictly be regarded as being an exact reflection of the evaporation rate of free water from the body.

That this compensating activity does not take place in the case of the tick appears from the following experiment. Groups of approximately fifty unfed nymphs each were weighed and exposed at 25° C. and humidities of 70, 50, 30 and 10 per cent. They were left until death supervened, and were then dried over anhydrous P_2O_5 at 50° C. until their dry weights became constant. As a control, the dry weight of a group of fifty, which had been kept at 90 per cent. R.H., was obtained by the same method. In Table III the original and dry

Table III. *Showing the ratio of dry weight to original weight in groups of fifty nymphs kept at different relative humidities at 25° C.*

		Ixodes ricinus nymphs		
Group	R.H.	Original weight in g.	Dry weight in g.	Dry weight as % of original weight
1	90	0·0121	0·0064	52·9
2	70	0·0105	0·0059	56·2
3	50	0·0122	0·0064	52·5
4	30	0·0132	0·0065	49·3
5	10	0·0121	0·0067	55·4

weights are given, and in the last column the ratio of dry weight/original weight is given. It will be seen that the ratio is practically constant for all humidities. Therefore, since the nymphs at the lower humidities lost weight before dying, the ratio of dry weight to total weight at death must have been different to that obtaining at 90 per cent., where there is no appreciable loss of weight of fasting ticks over a period of days. In other words, loss of water in the tick is not compensated by increased destruction of tissue to produce metabolic water, and loss of weight is therefore a direct indication of the rate of evaporation of water from the body.

The method adopted in this and the subsequent weighing experiments was as follows. Small metal capsules were prepared, open at one end and having the other end closed with fine-mesh wire gauze. The ticks were placed in these and the open end closed with lead foil, held in place by a thin rubber band. The containers were then weighed, an analytical balance being used, which weighed to 10^{-4} g. Since the average weight of a nymph is approximately 2×10^{-4} g., it was necessary, in order to get significant results, to weigh groups of fifty or more. Each container was suspended by a looped string inside the appropriate humidity system and weighed daily. At the end of the experiment the containers minus the ticks were weighed, and the weights of the ticks calculated. Since the container consisted entirely of metal, with the exception

of the thin rubber band, there was no variable introduced by hygroscopy of organic material, as would have been the case had cotton-wool plugs or muslin gauze been used.

Groups of nymphs of the same previous history were exposed to 25° C. and different humidities. This temperature was chosen because, as will be shown later, it is near the optimum temperature range for the unfed tick and, consequently, the heat-lethal effect may reasonably be expected to be very slight. Three experiments were carried out, at different times, and Table IV shows the daily losses in weight at the different humidities in each experiment. Each weight loss has also been expressed as a percentage of the original weight.

Table IV. *Daily loss of weight of nymphs at 25° C. and several humidities.*

R.H. %	Group	Original weight	Day 1		Day 2		Day 3		Day 4		Day 5		Day 6		Total weight loss %
		g.	g.	%	g.	%	g.	%	g.	%	g.	%	g.	%	
70	1	0·0105	0·0010	9·5	0·0008*	7·6	0·0009	8·6	0·0009	8·6	0·0008	7·6	—	—	30·5
	2	0·0112	0·0014	12·5	0·0001	0·9	0·0004	3·6	0·0005	4·5	0·0007	6·3	0·0008	7·1	34·9
	3	0·0099	0·0013	13·1	0·0006*	4·0	0·0008	8·1	0·0006	6·1	0·0006	6·1	—	—	33·4
	Total	0·0316	0·0037	11·7	0·0013*	4·1	0·0021	6·6	0·0020	6·3	0·0021	6·6	0·0008	7·1	—
50	1	0·0122	0·0010	8·2	0·0011	9·0	0·0011	9·0	0·0008	6·5	—	—	—	—	32·7
	2	0·0103	0·0013	12·6	0·0003	3·0	0·0009	8·7	0·0006	5·8	—	—	—	—	30·1
	3	0·0109	0·0009	8·3	0·0003	2·8	0·0006	5·5	0·0005	4·6	0·0007	6·4	—	—	27·6
	Total	0·0334	0·0032	9·6	0·0017	5·1	0·0026	7·8	0·0019	5·7	0·0007	6·4	—	—	—
30	1	0·0132	0·0016	12·1	0·0014	10·0	0·0016	12·1	—	—	—	—	—	—	34·8
	2	0·0097	0·0011	11·3	0·0010	10·3	0·0011	11·3	0·0010	10·3	—	—	—	—	42·9
	3	0·0107	0·0015	14·0	0·0015	14·0	0·0012	11·2	—	—	—	—	—	—	39·2
	Total	0·0336	0·0042	12·5	0·0039	11·6	0·0039	11·6	0·0010	10·3	—	—	—	—	—
10	1	0·0121	0·0022	18·1	0·0021	17·3	—	—	—	—	—	—	—	—	35·4
	2	0·0110	0·0017	15·5	0·0016	14·5	0·0015	13·6	—	—	—	—	—	—	43·6
	3	0·0108	0·0022	20·4	0·0012	11·1	—	—	—	—	—	—	—	—	31·5
	Total	0·0339	0·0061	18·0	0·0049	14·5	0·0015	13·6	—	—	—	—	—	—	32·5

* The weights marked with an asterisk are increments over the previous day's weight.

Each unit was discontinued when over 50 per cent. of the ticks had died. It will be seen that death occurred when approximately one-third of the original weight had been lost.

At 70 per cent. relative humidity there was in each experiment an unexpected interruption of weight loss on the second day, in two cases amounting to an actual increment. Thereafter, weight was lost steadily until death occurred. The regularity of this result over the three experiments precludes the possibility of its being due to an error in weighing (see Fig. 6). The possible significance of this observation will be discussed later.

If the rate of loss of water is proportional to saturation deficiency, the product of the percentage loss and the reciprocal of the deficiency for each humidity should be a constant (K). In Table V the daily losses, calculated from the total of the three experiments, have been divided by the saturation deficiency. It appears that for humidities of 50 per cent. and less, rate of loss of water is related to this humidity measure, the K values at the different relative humidities agreeing within the limits 0·43–0·84. At 70 per cent. R.H., however, the relation breaks down, the K value ranging from 0·89 to 1·65. (The second day, on which the interruption of weight loss occurred, has been omitted from the calculation.) It would appear from the high value for K at 70 R.H. that the

nymph loses relatively more water into air 70 per cent. saturated than into drier air. A similar finding has been recorded by Buxton (1930) for the meal worm, and by Mellanby (1932) for the bed-bug.

To test whether the relation under adverse moisture conditions held for a range of temperatures, a group of seventy-five nymphs was exposed to 20° C. and 30 R.H., and the daily weight loss calculated. The original weight of the group was 0·0142 g. The daily loss in grammes, the percentage of the original weight lost and the K value were as follows:

Day	g.	%	K
1	0·0016	11·27	0·92
2	0·0015	10·59	0·86
3	0·0010	7·04	0·57
4	0·0010	7·04	0·57

These values for K are in fairly close agreement with the values obtained at 25° C. (0·43–0·84); that is, under highly unfavourable conditions of humidity, rate of loss of water is approximately proportional to saturation deficiency, both at different saturation deficiencies for one temperature, and at different temperatures.

Table V. *Showing the ratio of weight loss to saturation deficiency for nymphs at 25° C. and several humidities.*

R.H.	70	50	30	10
Day 1	1·65	0·81	0·75	0·84
2	—	0·43	0·70	0·68
3	0·93	0·66	0·70	—
4	0·89	0·48	—	—
5	0·93	—	—	—
6	1·00	—	—	—

Since in two different types of experiment the survival of nymphs had been found to be unexpectedly short in completely dry air (Figs. 4, 5), the rate of water loss under such conditions was examined. A group of seventy-five nymphs, weighing 0·016 g., was exposed over anhydrous phosphorus pentoxide at 20° C. Since, even at this temperature, 100 per cent. deaths occur in 2 days (Fig. 3), only 1 day's weight loss could be obtained. The loss was 0·0051 g., *i.e.* 31·7 per cent. of the original weight. This divided by the saturation deficiency (17·54 mm.) gives a K value of 1·8. Thus, here again, we have evidence that under completely dry conditions nymphs lose water at a relatively greater rate than at higher humidities. A tentative explanation of this has been advanced earlier, viz. that under such extremely adverse conditions the nymph is unable to close its spiracles.

Table VI shows the rate of loss of water of larval clusters at 25° C. and different humidities. In this case also, loss of water appears to be roughly proportional to saturation deficiency at the lower humidities—$K = 0{\cdot}80$–$1{\cdot}09$—but the proportion breaks down at 70 per cent., K ranging from 0·68 to 1·82. This experiment is, however, extremely rough, since the larva is so small that clusters were used, and the end-point had to be determined arbitrarily, since

once the container was opened, the larvae could not be enclosed again, being both extremely active and, also, on account of their minute size, difficult to seize without injury.

Although both the larvae and nymphs at certain humidities appear to lose water at rates proportional to the saturation deficiency, yet the larva loses actually a greater proportion of its water content per day at a particular humidity than the nymph. This further supports the hypothesis that the nymph possesses some mechanism whereby, under unfavourable moisture conditions, the rate of loss of water is reduced.

The possession of such a mechanism may be associated with the unexpected results obtained with nymphs at humidities only slightly unfavourable. It is of interest to compare the rate of water loss at 70 per cent. saturation and different temperatures. Fig. 6 illustrates the rates of loss at 20, 25 and 30° C. The

Table VI. *Loss of weight of larvae at 25° C. and several humidities.*

R.H.	70				50				30			
S.D.	7·1				11·9				16·6			
Day	Weight of larvae	Loss of weight per day	% of original weight	*K*	Weight of larvae	Loss of weight per day	% of original weight	*K*	Weight of larvae	Loss of weight per day	% of original weight	*K*
0	0·0210	—	—	—	0·0222	—	—	—	0·0210	—	—	—
1	0·0200	0·0010	4·8	0·68	0·0201	0·0021	9·5	0·80	0·0172	0·0038	18·1	1·09
2	0·0173	0·0027	12·9	1·82	0·0185	0·0026	11·2	0·94	0·0141	0·0031	14·8	0·89
3	0·0153	0·0020	9·5	1·34	—	—	—	—	Dead	—	—	—
4	0·0128	0·0025	11·9	1·68	Dead	—	—	—	—	—	—	—
5	Dead	—	—	—	—	—	—	—	—	—	—	—
6	—	—	—	—	—	—	—	—	—	—	—	—
7	—	—	—	—	—	—	—	—	—	—	—	—

interruption of weight loss at 25° C. has already been commented on. The numbers indicate the individual results of the three experiments; the curve joining the circles is based on the average loss per day. At 20° C. a relative humidity of 70 is only very slightly unfavourable; the rate of loss, rapid at first, decreases later. At 25° C. there would appear to be an early abortive effort at compensating for evaporation, after which, loss proceeds steadily, while at 30° C., at which temperature 70 R.H. is distinctly unfavourable, loss of water proceeds evenly right from the commencement. As has been said, the irregularities may possibly be connected with the possession by the nymph of some method of conserving water. Thus, there would appear to be no interruption of loss at 25° C. and 70 per cent. in the case of the non-tracheate larvae. No explanation can, however, be offered at present of the physiological principles underlying the phenomena.

In this connection it is of interest to note that the rate of water loss in the larvae, in so far as the results can be relied on, is greater, relatively speaking, into damp air than into dry, yet the larva has no tracheal system.

(*d*) *Saturation deficiency, loss of water and survival*

Buxton (1931, 1932) has collected a mass of evidence dealing both with loss of water and with survival, and has shown that, with certain limitations,

saturation deficiency governs the loss of water of fasting insects of several species. Although recognising that exceptions exist, he suggests that this applies to the majority of insects. It has been shown above that this relationship applies also to the arachnid, *Ixodes ricinus*. It is of interest to consider to what extent Buxton's generalisation applies to survival. It has been seen that the relation of saturation deficiency to water loss at one temperature breaks down both under conditions of extreme aridity and under conditions only slightly unfavourable, so, therefore, its relation to survival also must be subject

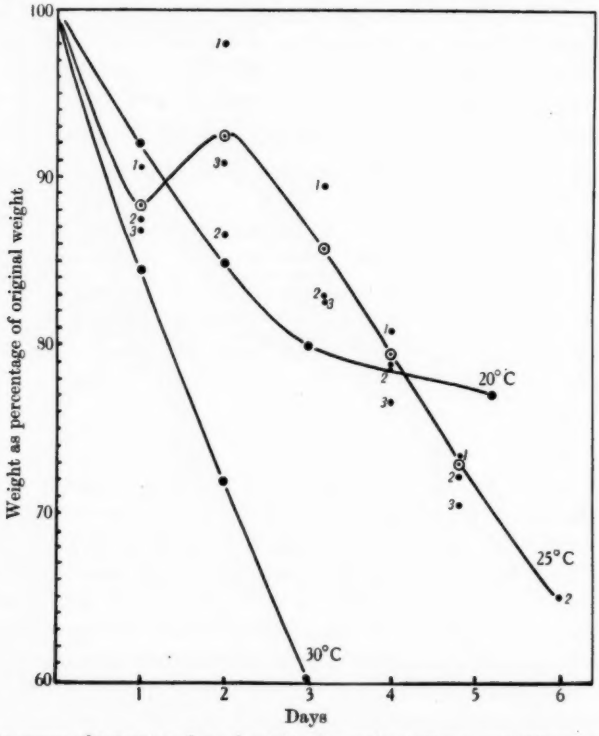

Fig. 6. The curves of percentage loss of weight of nymphs at 70 R.H. and different temperatures. The curve for 25° C. is based on the average of three experiments; the individual results are indicated by numbers.

to these limits. Even within these limits, the duration of survival does not maintain a fixed relation to rate of water loss, so the application of the relationship to survival must be further limited. Thus, at temperatures over the optimum, there is an increasing heat-lethal effect with increasing temperature. As has been shown above, there is also at such temperatures an increasing heat-lethal effect with increasing humidity. It follows, therefore, that Buxton's generalisation applies to survival of the tick only within strictly limited conditions both of humidity and temperature. Since humidities only slightly un-

favourable are without these limits, the practical utility of this generalisation in so far as the tick is concerned is very much reduced. Thus, since at conditions in the neighbourhood of the biological limit for the tick the limiting humidity for survival is not expressed by a single saturation deficiency, even over a narrow range of temperature, the law cannot be used in assessing the suitability of different climates or seasons, or in mapping the geographical distribution of the species.

IV. INFLUENCE OF TEMPERATURE ON ACTIVITY

In the preceding sections the limits of climatic conditions for indefinite survival of the unfed tick have been defined, and the duration of survival at conditions beyond these limits discussed. It is now necessary to consider which conditions, within this survival range, constitute the optimum for activity, and which, if any, produce responses conducive to host parasitisation, by affecting the relative accessibility of parasite and host.

It is clear that temperature is the important variable to be considered. The survival limits embrace a wide range of temperature and only a narrow humidity field. Further, changes in the moisture equilibrium of the air, within the narrow limits permissible, would not be expected to produce immediate responses, but rather to take effect through their prolonged application altering the water content of the body. It has already been shown (MacLeod, 1932) that tick activity can be correlated with air temperature, whereas there is no apparent relation between activity and rainfall.

(*a*) *Rate of activity*

Several nymphs and adults were placed in a corked tube, the atmosphere of which was kept saturated by a moist cotton-wool plug. Within the tube was a strip of paper marked in tenths of an inch, so that a measure of the rate of movement of the ticks could be obtained. The tube was placed in a water-bath, the temperature of which was steadily raised, and the behaviour of the ticks noted.

Irregular results were obtained with the lower temperature limit for activity. Movement in one or both stages began at 11° C. in some experiments, and not until 14° C. in others, according to the rate at which the temperature was raised. When the temperature was raised fairly rapidly, movement became general in both stages at 13° C. At 40° C. the stage of excited movement began, marked excitement being exhibited at 45° C. At 47° C. there was a slowing down of activity, and many of the adults became comatose. At 49° C. heat stupor was general in both stages, but recovery took place on cooling. Raising the temperature to 51° C. resulted in death. In Fig. 7 the rate of movement of nymphs at different temperatures is plotted, on a basis of seconds per unit (one-tenth of an inch). The reciprocal curve—units per second—is roughly sigmoid, suggesting that the velocity of movement may be related to temperature according to van't Hoff's formula for chemical reactions,

$Q_{10} = \frac{K_1}{K_0}\left(\frac{10}{T_1 - T_0}\right)$, if K be taken as the time required to travel a fixed number of units.

(b) Preferred temperature

A careful and detailed study of the response of this tick to different temperatures has been made by Totze (1933), who found that the preferred temperature depended on the previous conditions of temperature to which the

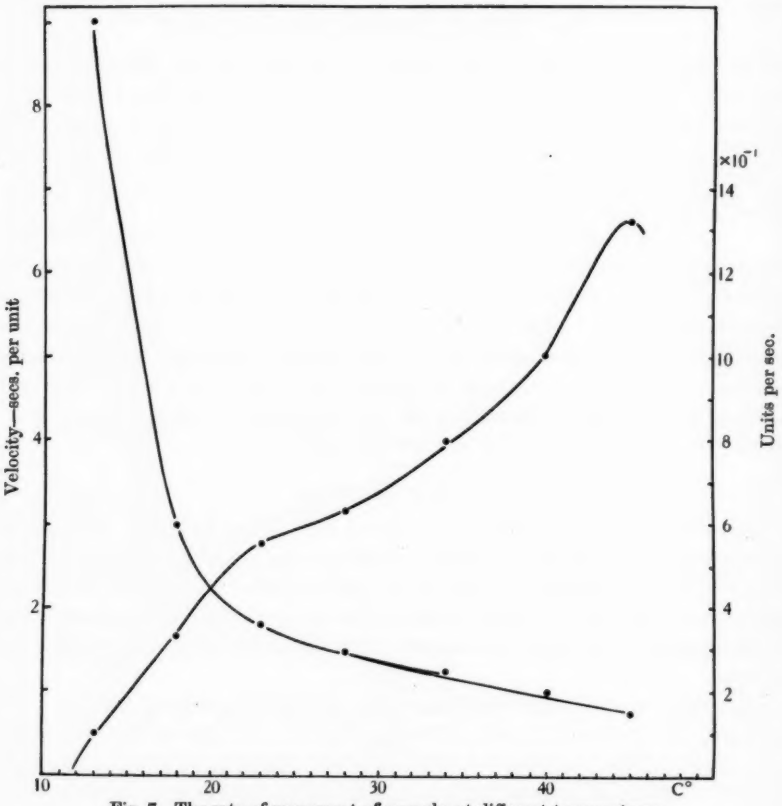

Fig. 7. The rate of movement of nymphs at different temperatures.

ticks had been exposed, varying from 15° C., in the case of ticks previously maintained at 10–12° C., to 19° C. (nymphs and larvae) and 23° C. (adults), when the previous temperature was 18–20° C. He found that ticks could be adapted to different preferenda by subjecting them for 2–2·5 hours to higher or lower preliminary temperatures.

In the experiments described below, the ticks used had been bred at room temperature (14–18° C.). The method adopted was as follows:

A copper strip, 30 × 3 in., was placed so that one end supported a block of

ice, while the other was warmed by a bunsen jet below it. There was thus a range of temperature along the copper strip, the limits of which could be varied by altering the size of the jet. It was found that if the ticks were confined to the plate by a paper flange, the complicating factor of thigmotropism was introduced, so ultimately the method adopted was to border the plate with a strip of vaseline impregnated with clove oil, contact with which ticks had been found to avoid. The enclosed area of the plate was marked off into sections, the temperatures of which were first obtained for each experiment as follows. A small pool of water was placed on each section. After half an hour the bulb of a thermometer was placed in the water, and an approximate index of the surface

Table VII. *Preferred temperature for unfed nymphs.*

Temp. ° C. ...	7–10	11–13	14–17	18–21	22–25	26–30
	No. of ticks in each section					
Exp. I	10	4	13	3	1	1
II	5	0	20	2	4	5
III	13	3	22	5	0	0
IV	17	9	28	3	5	0
Average	11	4	21	3–4	2–3	1–2

temperature of the section was thus obtained. The ticks were distributed evenly along the plate, and left to choose whichever section was most suitable. The apparatus was roofed over with black cloth to exclude light. The ticks were left on the plate for about 1 hour, and then the result of the experiment was taken. Table VII records the results of the experiments with nymphs. Those in the coldest section were probably inactivated by the cold and remained motionless. If this section be ignored, it would appear that the temperature preferred by the nymphs was 14–17° C. This agrees with Totze's finding of 17° C. for nymphs reared at 14–18° C. In two similar experiments with larvae, the preferred temperature range was found to be 16–17 and 18–21° C. respectively. Totze records the larvae as behaving similarly to the nymphs.

From these and Totze's results, one may assume that the preferred temperature range of the tick lies between the limits of 14 and 23° C.

V. THE PHOTOTROPIC RESPONSE AT DIFFERENT TEMPERATURES AND HUMIDITIES

The evidence with regard to phototropism of ticks in general is conflicting. Thus, Hindle and Merriman (1912) found that *Argas persicus* was negatively phototropic in all stages. Similarly, Hunter and Hooker (1907) record that the female of *Margaropus annulatus*, an eyed tick, is strongly negative in its heliotropic response. Yet, in the eyeless *Ixodes ricinus*, Olenev (1927) and Totze (1933) record a positive phototropism for the unfed tick. Totze describes fully his methods and results, which indicate that all the unfed stages are positively phototropic, and that there is an optimum intensity of light above and below which the ticks are less marked in their response. The writer has been entirely unable to confirm this result.

The response of the tick to light was tested in three different ways:

(1) A sheet of white paper was placed on a hot plate, the temperature of which was electrically controlled. This was placed near a window, ticks liberated on to it, and their paths traced. In all instances they were negatively heliotropic.

(2) Ticks were distributed evenly inside long glass test-tubes, which were placed at different positions on a copper plate. This was supported on trestles, with a row of gas jets under one end. There was thus a temperature gradient along the plate, the limits of which could be adjusted by altering the flame of the jets. This method prevented any lateral movement, and gave a similar result to the first method.

Following on this work, a routine method of manipulation of ticks, depending on their negative phototaxis, has been successfully used for the last few years.

Subsequently to the above work, Totze's work was published, and, since his results were in direct contradiction to the writer's findings, a careful study of the subject was made, under properly controlled conditions of temperature, humidity, and radiant heat.

(3) A water-jacketed thermal cabinet was used for controlling temperature; different degrees of humidity were obtained by the use of potash solutions. A solution of the required concentration was placed in a large petri dish, over which was then stretched a piece of fine muslin gauze, held taut by a rubber band. On this the ticks were placed. Another glass dish of the same diameter was inverted over the first, and thus the ticks were enclosed in an air space, the relative humidity of which depended on the concentration of the solution below it. The whole was placed in the cabinet, the temperature of which had been brought to the required degree, and left for an hour, so that the temperature and humidity in the system might attain to equilibrium. Light from a 60 watt lamp, 1 ft. away, was then admitted through a glass pane in one side of the cabinet, and the reaction of the ticks observed through a sliding panel in the roof of the cabinet. The light, before entering the cabinet, was passed through a bath of 1 per cent. ferric sulphate solution, which absorbed the heat rays.

In all the experiments, it was observed that the ticks exhibited less a definite response than a general tendency. Thus, some individuals remained indifferent for some time, wandering at right angles to the light rays; others travelled towards the light source for some distance; but all eventually tended to congregate at the side of the vessel farthest away from the light.

Both adults and nymphs were tested, and experiments were carried out at each of the following temperatures and humidities, the result being in all cases the same—a negative phototropic response.

Temperature °C.	R.H.				
11	100	90	—	—	—
14	100	—	—	—	—
16	100	90	70	50	—
17	100	90	—	—	—
22	100	—	70	—	—
25	100	90	70	50	—
28	100	90	70	50	30
30	—	—	70	—	30
33	—	—	70	50	30
35	100	90	70	50	30
38	—	90	70	50	—
40	100	—	70	50	30

In Totze's experiments also, radiant heat was absorbed by a suitable solution, so the difference in the results cannot be due to positive thermotaxis introducing a source of error, and therefore remains unexplained.

Gorged nymphs and larvae were also found to be negative in their reaction.

VI. THE INFLUENCE OF TEMPERATURE ON GEOTROPIC RESPONSE

The following method was used for studying the reaction of the tick to the stimulus of gravity at different degrees of temperature. A glass cylinder of 1 in. diameter was placed upright in a copper water-bath, so that it projected about 9 in. above the level of the bath. This and the bath were filled with water, the temperature of which could be kept at any desired degree by the regulating mechanism of the bath. A number of special tubes were prepared by removing the flanges from test-tubes and holding each couple together, mouth to mouth, by a strong, narrow rubber band. Each tube was thus domed at both ends, and this eliminated the possibility of complicating modifications from thigmotropic stimuli. One end of each containing tube was ground down until the glass was perforated. Over the hole a piece of muslin gauze was pasted. The continuity of the inner surface of the tube was thus maintained, while the muslin allowed of the humidity in the tube being regulated.

By placing one of these tubes, containing ticks, in the exposed part of the glass cylinder, it was possible to expose the ticks to any desired temperature in daylight. A weight attached to the lower end of the tube kept it practically submerged in the water, the perforated end only being above the surface. The narrow diameter of the cylinder prevented the tube from leaving the upright position. Similarly, to expose ticks in darkness to any desired temperature, the containing tube was placed inside the copper water-bath with an attached weight to keep it upright, and submerged except for the perforated end.

Fig. 8 shows the geotropic response of unfed larvae at different temperatures, with and without the presence of daylight. A closed circle indicates a marked tendency, involving all, or the great majority of, the larvae; an open circle indicates a less definite but appreciable tendency. Circles on the left of the vertical lines indicate a positive geotropic response; on the right, a negative response. A limited number of experiments with unfed nymphs suggests that the response of this stage is similar to that of larvae.

It will be seen from the figure that, in darkness, larval ticks are positively geotropic at temperatures below 12 and above 30° C. At temperatures between these limits they are negatively geotropic. Under the influence of daylight the intensity of response is greater, and the range of temperature for which the tick is negatively geotropic is more clearly defined as 14–24° C.

It has been shown previously that ticks are inactive at temperatures below 10° C. From the above result it might be assumed that at temperatures of 10–14° C., although active to a greater or lesser extent, they tend in nature to remain at the roots of vegetation. Again, at temperatures of over 24° C., the ticks would be expected to forsake the vegetation for the surface of the ground.

A similar inhibiting effect of high temperatures on negative geotropism has been found by Fraenkel (cited by Uvarov, 1931) in the case of the desert locust, the hoppers of which climb the plants only at temperatures below 30° C., while above it they remain on the ground.

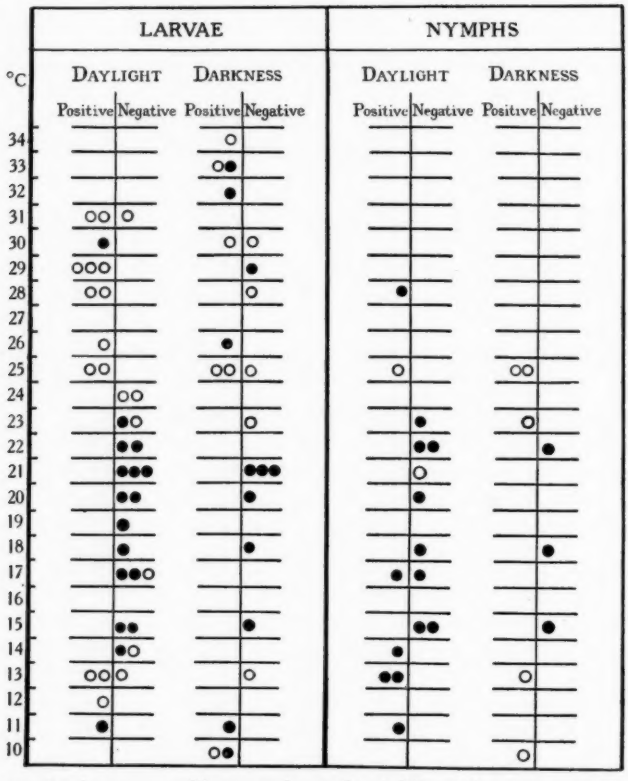

Fig. 8. The geotropic response of larvae and nymphs at different temperatures. Closed circles indicate a definite response; open circles a less definite but appreciable response. Circles on the left of the vertical lines indicate a positive, and on the right, a negative response.

VII. Discussion

The most marked feature of the climatic requirements for survival of the unfed *Ixodes ricinus* is the high degree of humidity necessary. Saturated air appears to have no adverse effect on vitality, a markedly different result from that obtained by Cunliffe (1921, 1922) for *Ornithodorus moubata* and *savignyi*, ticks of dry climates, which are adversely affected by excess moisture. The lower limit of humidity for survival of *Ixodes ricinus*, throughout the limits of temperature normally obtaining in nature in this country, is fairly well represented by a relative humidity value between 70 and 80.

The tick shows considerable powers of resistance to freezing, if the temperature be lowered slowly so as to allow of the development of a cold hardiness; the upper temperature limit for survival, on the other hand, is not very high.

Within these survival limits of temperature and humidity, the conditions necessary to allow of the tick's gaining access to hosts are those which permit of normal activity. It has been shown that the tick becomes active at temperatures of 11° C. and over. If the preferendum temperature be taken as the most favourable for normal activities, the limits of 14–23° C. are indicated.

In Nature, the unfed tick is found on the tops of vegetation, whence it is rubbed off by passing hosts. The second condition, therefore, under which access to hosts is favoured is that which induces a climbing habit. The tick is negatively geotropic in daylight within the temperature limits of 14–24° C. Within this range they will tend to congregate on tops of grass, rushes and shrubs. The limiting factor for host parasitisation, as opposed to that for survival, would therefore appear to be primarily temperature, opportunities for attachment to a host being most favourable at temperatures of 14–24° C.

The various experiments with unfed ticks have been carried out at all seasons of the year, as have also the processes of feeding and development. In no case has any indication been obtained of the overwintering torpor of unfed ticks described by Totze (1933), or of gorged ticks, Falke (1931). Falke's hypothesis has been discussed in my previous paper. Totze mentions a recognisable dullness as the sign of approaching torpor, which lasts from mid-September to the beginning of April, and which is not affected by altering the temperature or humidity. This could be possible only if there occurred in the tick an auto-intoxication from development, necessitating a seasonal diapause for physiological purification, comparable to the asthenobiosis of heterodynamic insects (Roubaud, 1922). The fact that ticks have been fed, and the gorged stages hastened through development at favourable temperatures in winter and summer, with no difference in the time required for metamorphosis (MacLeod, 1934), is sufficient evidence that in this country, at any rate, such seasonal variation in metabolic activity does not exist.

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(*MS. received for publication* 23. vi. 1934.—Ed.)